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ELF Communications System Ecological Monitoring Program: Summary of 1990 Progress

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A long-term ecological monitoring program is being conducted to monitor for possible electromagnetic effects that operation of the U.S. Navy's ELF Communications System might have on resident biota and their ecological relationships. Monitoring studies were selected through a peer-reviewed, competitive bidding process in mid-1982, and work on most studies began in late summer of that year. Preliminary activities of the program consisted of site selection, characterization of critical study aspects, and validation of assumptions made in original proposals. Data collection for studies at the Naval Radio Transmitting Facility (NRTF)-Clam Lake, Wisconsin was completed, as scheduled, during 1990. Data collection for studies at the NRTF-Republic, Michigan is planned to continue through 1992. This report summarizes the progress of the monitoring program during 1990.

To date, investigators conclude that no effects have occurred on biota exposed to EM Fields produced by either a fully operational or an intermittently energized ELF transmitting facility.

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FOREWORD

The U.S. Department of the Navy is conducting a long-term program to monitor for possible effects that operation of the Navy's Extremely Low Frequency (ELF) Communications System might have on resident biota or their ecological relationships. The program is funded by the Space and Naval Warfare Systems Command through a contract to IIT Research Institute (IITRI). IITRI provides engineering support to the program and coordinates the efforts of ecological study teams. Monitoring projects are being conducted under subcontract arrangements between IITRI and university investigators.

This report summarizes the activities of the ELF Communications System Ecological Monitoring Program during 1990. The information presented was derived from other, more detailed technical reports of ecological findings and electromagnetic exposures.[1,2]

Since the inception of the program in 1982, IITRI has annually compiled subcontractor reports of efforts and findings,[1, 3-10] documented engineering support activities,[11-17] and summarized the progress of the program.[18-25] Subcontractor reports have been peer reviewed, and all were submitted (unedited by the Navy or IITRI) to the National Technical Information Service for unlimited distribution. Investigators have also related their findings as presentations to scientific societies and as articles in peer-reviewed journals. Appendix A lists the presentations and publications.

Respectfully submitted,
IIT RESEARCH INSTITUTE

J. E. Zapotosky, Ph.D.
Program Coordinator

Approved:

R. D. Carlson, Director
ELF EM Compatibility Assurance

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GLOSSARY AND ACRONYMS

AIBS	American Institute of Biological Sciences.
ANOVA	analysis of variance, a statistical method for partitioning the total variability affecting a set of observations between the possible and statistically independent causes of the variability.
ANCOVA	analysis of covariance, a statistical method for determining whether the functional relationships described by two or more regression equations are the same; it is used when treatments are compared in the presence of accompanying variables that cannot be eliminated or regulated.
ATP	adenosine triphosphate.
BACI	before and after, control and impact, a statistical technique that compares the mean of the "before" differences between the control and impact (treatment) sites to the mean of the "after" differences between sites by using an unpaired t-test; also known as intervention analysis.
biomass	a quantitative estimate of the total mass of living organisms comprising all or part of a specified unit such as a population.
chi-square test	a statistical method for testing the degree to which observed frequencies or values differ from frequencies or values expected from the specific hypothesis being tested.
clone	an assemblage of genetically identical organisms derived by a sexual or vegetative multiplication from a single sexually derived individual.
clutch	the number of eggs laid at any one time.
control site	a location where parallel observations or experiments are carried out; they provide a standard against which a result can be compared. As used in this report, it is a location remote from the ELF Communications System, having 76 or 44 Hz EM intensities at least one order of magnitude less than at the matched treatment site.
dendrometer bands	a method of measuring plant growth by examining increases in trunk or stem diameter.
detritivore	an animal feeding on fragmented particulate organic matter.
diel	pertaining to a 24-hour period.

edge effect	the impact exerted by adjoining communities on the population structure within the marginal zone, which often contains a greater number of species and higher population density of some species than either adjoining community.
ELF	extremely low frequency, as generally used, refers to frequencies ranging from 0 to 300 Hz. As used in this report, it refers to operating frequencies of the Navy's ELF Communications System (i.e., 76 ±4 Hz, 44 ±4 Hz).
EM	electromagnetic.
evapotranspiration	the loss of water by evaporation from soil and transpiration from vegetation.
evenness	the apportionment of individuals among those species found in a given community.
fecundity	the potential reproductive capacity of an organism or population, measured by the number of gametes.
generation time	the average duration of a life cycle between birth and reproduction.
genetic diversity	a measure of the genotypic disparity within a population, the different forms of a gene occupying the same locus or loci.
guild	a group of species having similar ecological resource requirements and foraging strategies, and therefore having similar roles in the community.
herbaceous plants	plants that have stems that are not secondarily thickened or lignified and that die down annually.
litter	recently fallen plant material that is only partially decomposed and in which the organs of the plant are still discernible, forming the surface layer on some soils.
MDD	minimum detectable difference, the magnitude of the smallest change that can be perceived for a given sample size and parameter variance.
MSU	Michigan State University.
MTU	Michigan Technological University.
mycelium	the mass of filamentous hyphae that comprises the vegetative stage of many fungi.
mycorrhiza	the close physical association between a fungus and the root system of a plant.
NAS	National Academy of Sciences.

NRTF	Naval Radio Transmitting Facility.
nymph	a stage in the development of some arthropods that occurs between hatching and the reorganization involved in attaining the adult stage.
oogenesis	the formation, development, and maturation of female gametes or eggs.
periphyton	a community of plants, animals, and associated detritus adhering to and forming a surface coating on submerged objects.
phenology	the study of temporal aspects of recurrent natural phenomena.
pupa	the life cycle stage of an insect during which the larval form is reorganized to produce the final, adult form; commonly an inactive stage enclosed within a hard shell.
regression analysis	in statistics, the estimation of the relationship between one variable and one or more other variables, by expressing one in terms of a linear or more complex function of the others.
ROW	right-of-way; a cleared corridor for the location of transmitter elements.
sham ROW	a cleared corridor on control sites that is used to duplicate possible effects from the antenna ROW on study variables.
significance	the probability that experimental results have not occurred by chance alone.
species richness	the absolute number of species in an assemblage or community.
t-test	a statistical method used for determining the significance of the difference between two means when the samples are small and drawn from a normally distributed population.
treatment site	a location where primary observations or experiments are carried out and where biota are exposed to 76 or 44 Hz EM intensities at least one order of magnitude greater than at the matched control site.
UMD	University of Minnesota-Duluth.

EXTREMELY LOW FREQUENCY (ELF) COMMUNICATIONS SYSTEM ECOLOGICAL MONITORING PROGRAM: SUMMARY OF 1990 PROGRESS

1. INTRODUCTION

1.1 PURPOSE

The purpose of the Ecological Monitoring Program is to determine whether electromagnetic (EM) fields produced by the Navy's ELF Communications System will affect resident biota or their ecological relationships.

1.2 ELF COMMUNICATIONS SYSTEM

The complete ELF Communications System consists of two transmitting facilities, one located in the Chequamegon National Forest in Wisconsin and the other located in the Copper Country and Escanaba River State Forests in Michigan (see Figure 1). The Wisconsin facility is designated as the Naval Radio Transmitting Facility (NRTF)-Clam Lake, and the facility in Michigan as the NRTF-Republic.

Each facility consists of three elements: (1) a transmitter connected by (2) long overhead wires (i.e., the antenna) to (3) buried ground terminals. Both the antenna and grounding elements are located in cleared rights-of-way (ROWs). The transmitters broadcast messages using ELF EM fields; these fields are the operational components of interest.

For the purposes of this report, EM exposure from the ELF Communications System has been divided into preoperational, intermittently operational, and operational phases. During the preoperational phase, biota received no EM exposure from the ELF Communications System. The intermittently operational phase began with the initiation of system testing; EM exposures during this phase were sporadic and at lower intensities than intended for a fully operational ELF System. The NRTF-Clam Lake was operating intermittently at the time of initiating the monitoring program in 1982; it reached a fully operational capability during the last quarter of 1985. In Michigan, intermittent operations began in late 1985; the NRTF-Republic became fully operational during October 1989. With full operational capability, EM exposures at both facilities are nearly continuous and at maximal intensity.

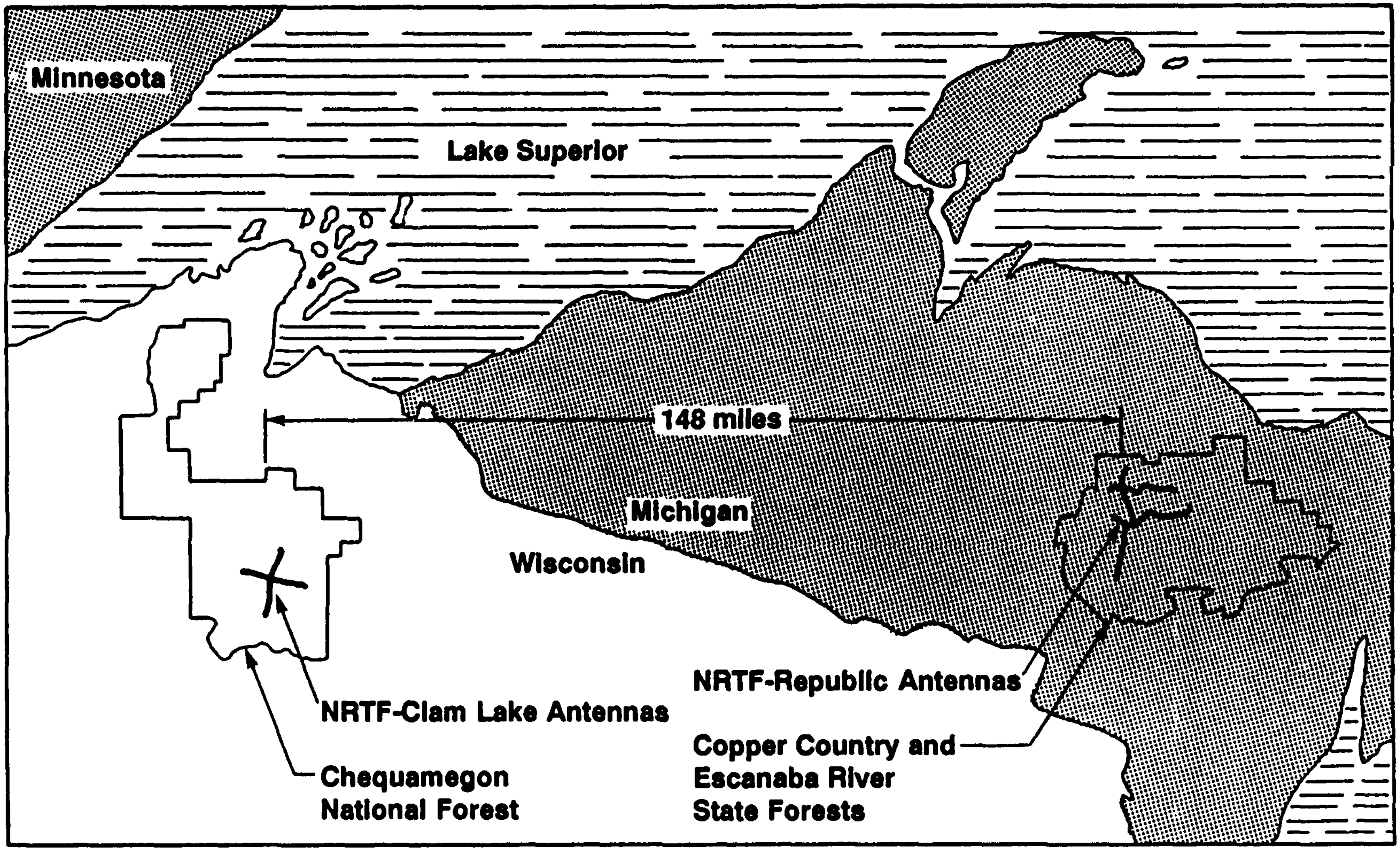

Figure 1. ELF Communications Facilities in Wisconsin and Michigan

1.3 ELF BIOEFFECT EVALUATIONS

Research on possible EM effects to biota from exposure to EM fields produced by an ELF Communications System began in 1969. Although ecological and wildlife studies were performed in the ensuing years, the major emphasis of most ELF System-related research was on laboratory investigations. In 1977, both the Navy and the National Academy of Sciences (NAS) examined the information produced by these studies as well as studies performed at other ELF frequencies. Specific research simulating planned operating conditions of the ELF System, as well as research at other ELF frequencies, indicated no acute bioeffects from exposure to ELF EM fields at planned operational intensities. The Navy and the NAS concluded that adverse effects to biota from the operation of the ELF System were unlikely. After reviewing the pertinent bioelectromagnetic research reported in open literature over the 1977-1984 period, the American Institute of Biological Sciences (AIBS) reached the same conclusion as the Navy and the NAS. Despite the unlikelihood of adverse effects, the Navy, and subsequently the NAS and AIBS, recommended that a program be conducted in the ELF Communications System area to monitor for possible changes to resident biota.

1.4 MONITORING PROGRAM DESIGN

In its 1977 environmental impact statement, the Navy outlined a preliminary plan for conducting a monitoring program at those sites approved for operation of the ELF Communications System. The initial design was developed from the results of laboratory research, input from state agencies, and recommendations made by the Navy and the NAS. These elements were later refined based on comments submitted in response to the Navy's draft environmental impact statement. A long-term program of *in situ* monitoring of biological and ecological variables was planned. Possible effects to pertinent biota were to be examined for by rigorous statistical analyses of spatial and temporal differences.

Study Organisms and Variables. The selection of general types of organisms for monitoring was based on their likelihood of being affected by EM fields and their ecological significance. Literature reports of EM effects, even though at higher intensities or at ELF frequencies other than those used by the ELF System, were used in the selection process. The importance of the organisms to the ecosystems present in the area also was considered. Upon completion, the program will have examined 16 general types of organisms dominant in the upland, wetland, and aquatic ecosystems present in the ELF Communications System area.

The principal criterion for selecting specific biota was their presence in sufficient numbers to ensure meaningful comparisons. Rare or endangered species have not been examined because of their low population sizes.

The program monitors for possible effects at several levels of biological organization. Organismal studies focus on the characteristics of the individual (e.g., behavior, physiology). Ecological variables address levels of organization more complex than the individual (i.e., populations, communities, and ecosystems). Population variables (e.g., density, fecundity, distribution) are important because they can reflect possible subtle effects to many individuals. Community and ecosystem variables (e.g., diversity, productivity, nutrient cycling) integrate the responses of many individuals and species.

An ecological approach has been taken to examine for possible effects to the disparate species present in the area. One limitation of this approach is that ecological characteristics are highly variable, so a substantial effect must be demonstrated in order for researchers to detect it. Organismal studies have been undertaken to focus on purported EM-sensitive parameters. Although narrower in scope than ecological studies, these studies are more statistically precise. Except for the completed studies of slime molds, every project in the program has coupled organismal studies with monitoring at ecological levels.

Study Sites and EM Exposure. The monitoring program uses a paired treatment and control site design to examine for possible effects of ELF EM fields on biological and ecological variables. Treatment sites are positioned close to the overhead wires and grounds of the transmitters, while control sites are located at a distance from these transmitter elements (see Figures 2 and 3). Such sites have essentially matched biotic and abiotic characteristics, but purposely dissimilar ELF EM exposures. Sites have been located so as to ensure that the intensity of ELF System-generated EM fields at the treatment site are significantly larger than those at the control site. Data collected at the control site represent the effects of natural environmental conditions, whereas data collected at the treatment sites represent the effects, if any, of natural conditions plus exposure to higher EM intensities relative to those at the control site.

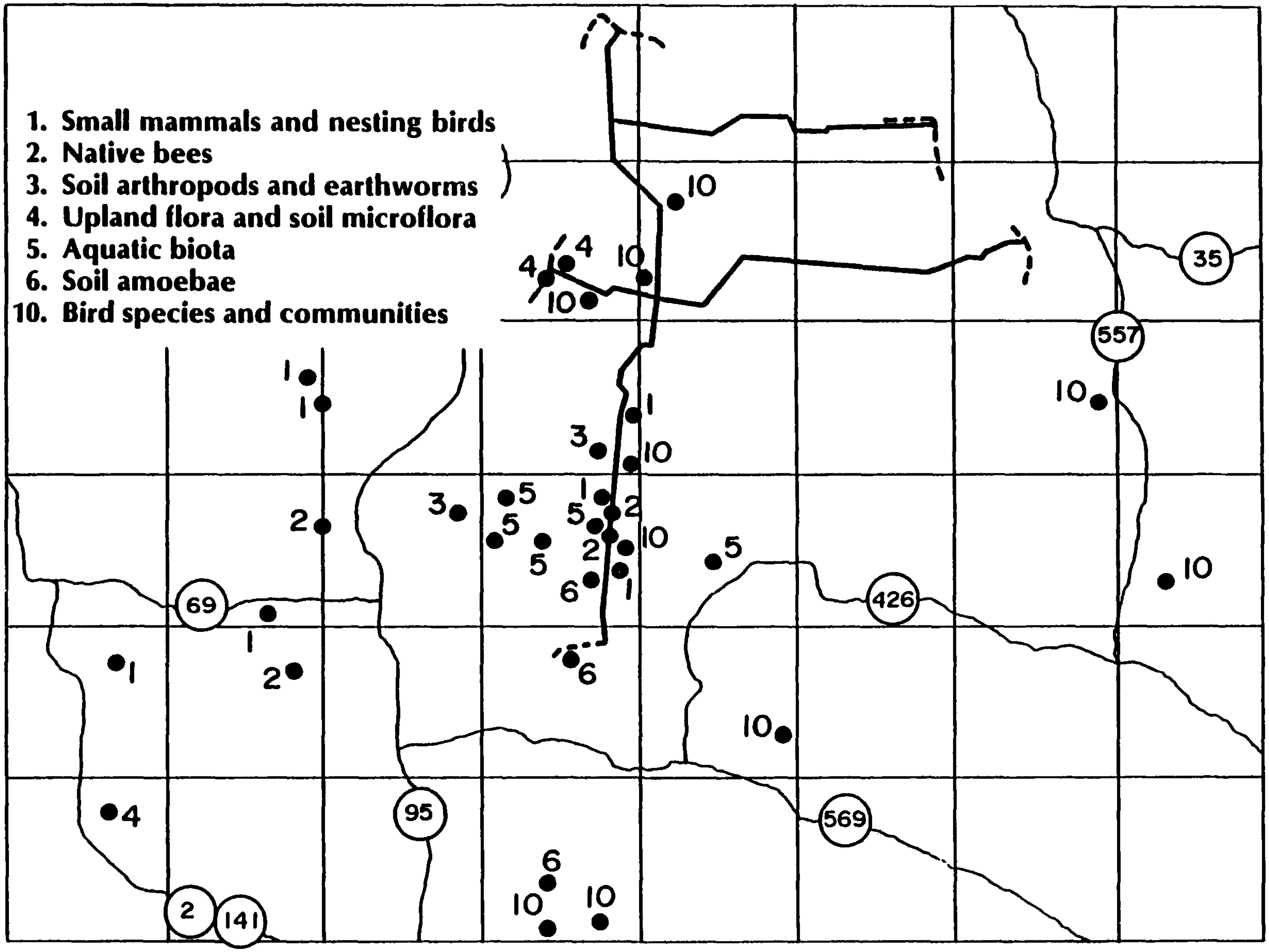

Figure 2. Field Sites for Michigan Ecology Studies

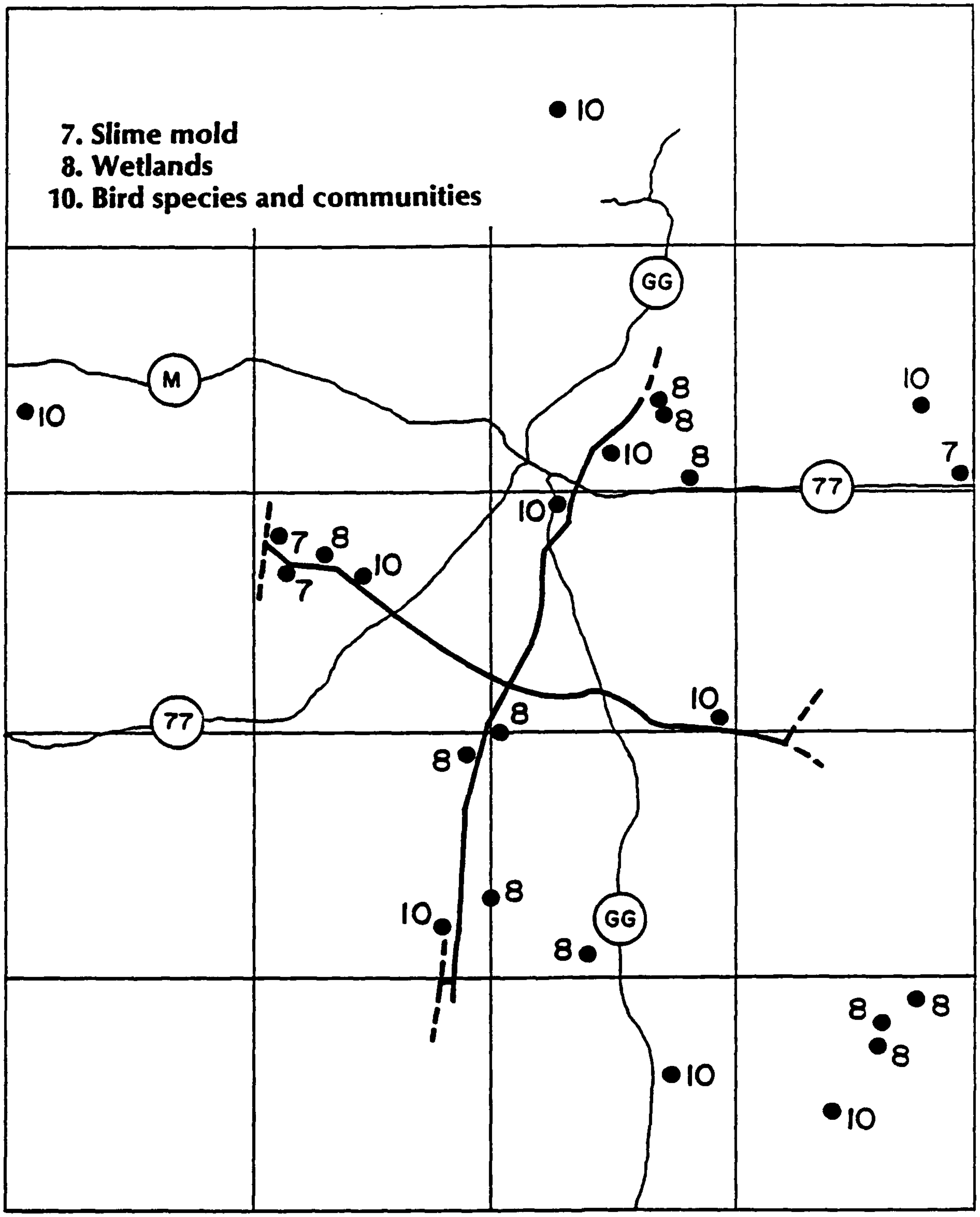

Figure 3. Field Sites for Wisconsin Ecology Studies

As multiyear studies are being performed, temporal comparisons of variables are also possible. Comparisons of data collected during the preoperational phase (no EM exposure) with data collected during the intermittent and operational phases of the NRTF-Republic have been made for Michigan studies. A preoperational data base does not exist for studies completed in Wisconsin; therefore, comparisons were primarily spatial.

Period of Performance. The period of performance for the program had to address several temporal aspects, including organismal generation times, the time when the antenna became fully operational, and non-ELF cyclic changes in variables. The schedules for studies in Michigan and Wisconsin are presented in Figures 4 and 5.

EM effects on resident biota, if any, will be subtle and therefore will not be expressed at the population or community levels for several generations. Long-lived animal species, in particular, have generation times longer than two years. If adults are less susceptible than younger individuals, one would anticipate a time lag until lack of recruitment of young individuals is reflected in processing rates (e.g., decomposition) and/or community composition (e.g., diversity). Thus, if ELF EM fields affect the development of the young in such species, possible effects may not be apparent for several years.

Early studies of the ELF Communications System lacked a preoperational data base, and were performed while the (then) Wisconsin Test Facility was intermittently energized at less than full power. In order to more properly examine for possible EM effects, biological and ecological data must be collected while the ELF System is fully operational. In the current program, two to four years of data were collected during the operational phase of the NRTF-Clam Lake, while at the NRTF-Republic more than three years of operational observations are anticipated.

1.5 PROGRAM DEVELOPMENT

Concurrently with approval to complete construction of the ELF Communications System, the Department of the Navy funded the Ecological Monitoring Program. Early in 1982, a competitive process was initiated to select subcontractors to participate in the program, and by mid-summer preliminary work began for studies of upland flora, soil microflora, soil amoebae, soil arthropods and earthworms, native bees, small mammals and nesting birds, aquatic biota, and slime molds. Unsolicited proposals for studies of wetland

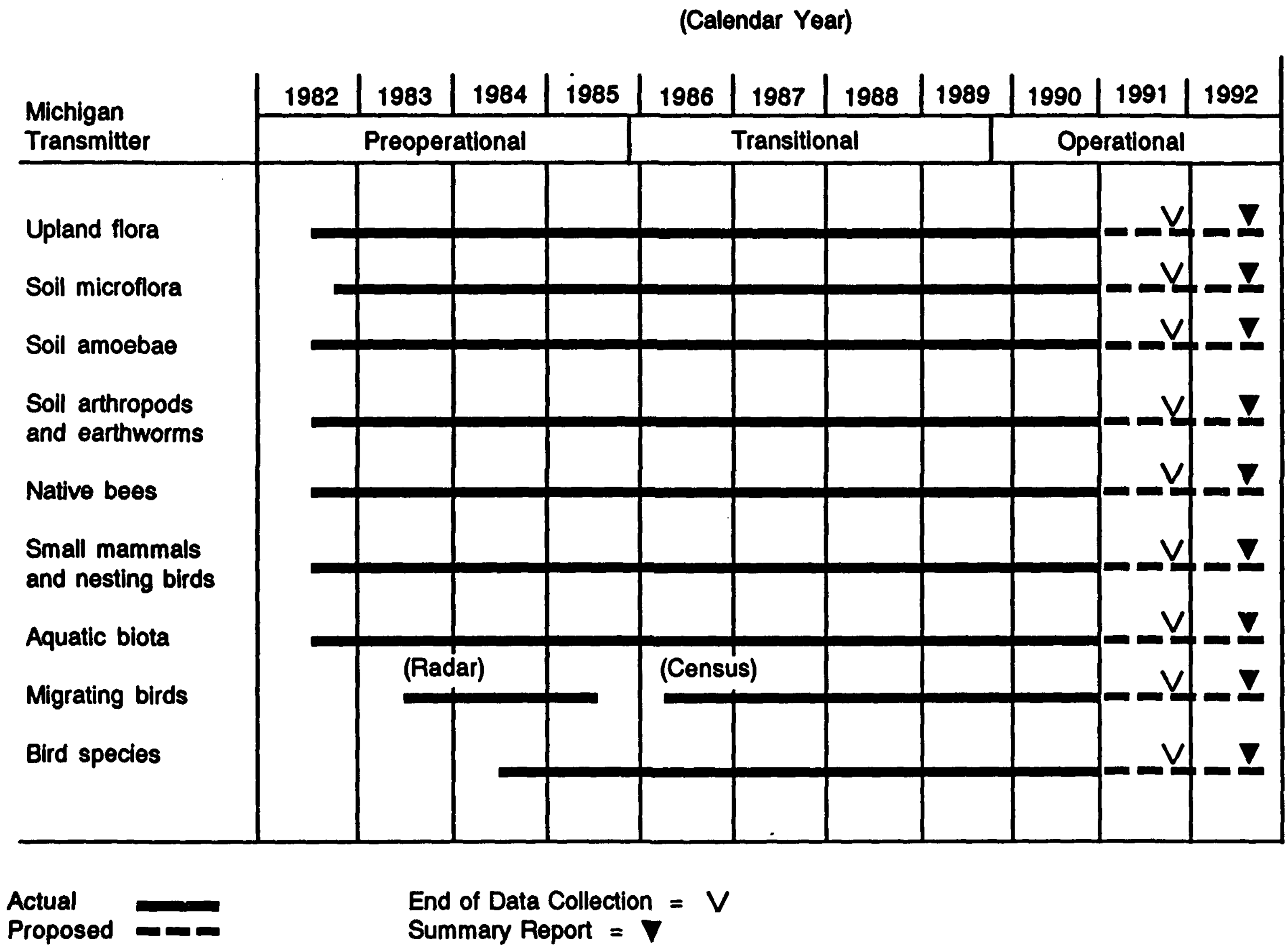

Figure 4. Schedule for Michigan Studies

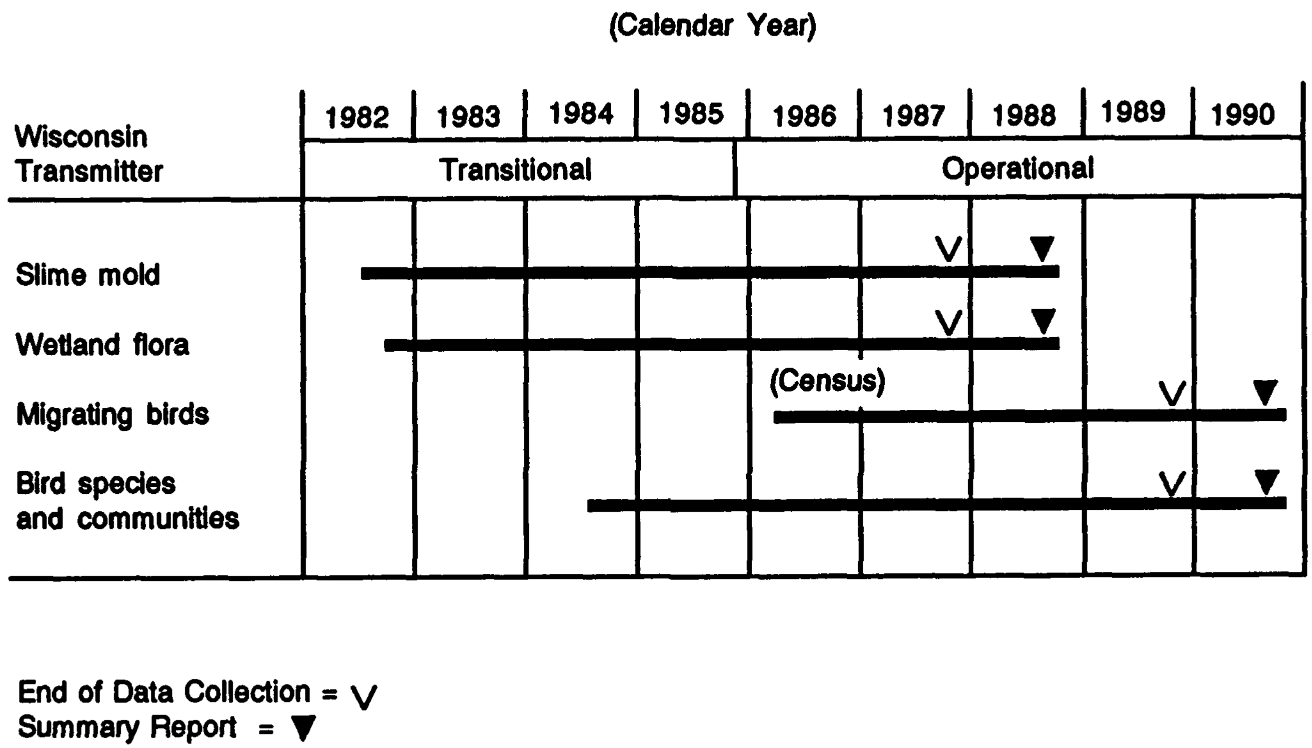

Figure 5. Schedule for Wisconsin Studies

flora and migrating birds were funded the following year. These latter two studies, together with the eight studies selected in 1982, constituted the program in 1983.

In both 1983 and 1984, peer reviewers expressed serious doubts that the then-ongoing radar studies of migrating bird disorientation could be used to successfully detect possible effects of the ELF Communications System. The radar study was replaced in 1986 with a census of migrating birds.

The major objectives of each study during the early years of the program were the selection of study sites, the validation of assumptions made in the proposals, and the characterization of critical study aspects. These objectives encompassed such activities as:

- identification of biota
- assessment of data collection protocols
- quantification of spatial and temporal patterns for each variable
- assessment of parameter variability

As these tasks were accomplished, increasing emphasis was placed on the collection of data and the refinement of statistical protocols.

In Wisconsin, studies of wetland flora and slime mold have been completed, and a summary report for each[26,27] has been distributed. Both studies found some significant differences between sites in the variables examined; however, the significant findings showed no consistent pattern among parameters, species, or sites. Temporal changes did not match energization of the transmitter, and in several cases significant findings were not supported by additional statistical analyses. ELF researchers concluded that there were no ELF EM bioeffects on wetland flora or mold metabolism.

Bird studies were performed in both Wisconsin and Michigan. Wisconsin studies were completed as scheduled during 1989 and showed some consistent, significant differences between sites for several bird population characteristics. Even though census transects were randomly selected, more coniferous, lowland habitat was found on treatment transects than on control transects. Significant differences between sites in bird community parameters have been attributed to the differences in habitat. A summary report[28] was distributed in early 1991. Collection of data on bird communities and species in Michigan will continue through 1991.

In Michigan, studies continued to collect data and further develop their statistical protocols during 1990. Several variables have shown significant differences on a temporal scale between the preoperational and operational phases of the NRTF-Republic; however, there were no corresponding significant differences between sites. The significant temporal differences have been attributed to a multiyear drought that started at the same time as intermittent operation of the facility in 1986. Other studies in Michigan have not shown spatial or temporal differences attributable to ELF EM fields.

Wildlife surveys near the NRTF-Clam Lake have also been performed by the U.S. Forest Service. Although not an intergral part of this program, the results of these surveys are generally applicable to the program objectives. Annual surveys of ruffed grouse, eagle, and deer populations were initiated in 1974, 1975, and 1982, respectively. No effects on these populations from the operation of the ELF Communications System were detected, and the studies were concluded after the 1986 surveys. A summary of protocols and data can be found in Reference 22.

2. BIOLOGICAL/ECOLOGICAL STUDIES

This section summarizes the progress for each of the eight studies that constituted the program during 1990. A more detailed presentation of study protocols, methodology, and progress is given in individual project reports.

The general types of biota being examined are used as subsection titles, while specific study elements are presented as underlined run-in titles in each subsection. In order to simplify presentation of statistical results, any difference described as "significant" had a significance level of 5 percent ($P < 0.05$).

2.1 UPLAND FLORA

Forest vegetation is the dominant biota in the ELF Communications System area. The production of organic compounds by vegetation and the subsequent degradation of these compounds make up the main method for the transfer of energy and nutrients to other organisms. Indeed, organic matter turnover and distribution are regarded as major determinants of the forest ecosystem structure. Because the production and distribution of organic matter have been shown to be measurably affected by anthropogenic factors, these processes and associated organisms are being monitored for possible effects from the ELF Communications System. Important aspects in the production of organic compounds by upland vegetation are presented here; those organisms and processes important in organic matter decomposition are addressed in Sections 2.2, 2.3, and 2.4.

In order to examine for possible changes in upland productivity and health, the following elements are being examined:

- growth rates of pine seedlings and established tree stands
- phenological events of trees, herbs, and mycorrhizal fungi
- numbers and kinds of mycorrhizae on red pine roots
- foliar nutrients and litter production
- insect damage, disease, and ambient environmental factors.

The experimental design for these studies is best described as a split plot in space and time. Two treatment sites are located so that one site is adjacent to an antenna element, and the other, adjacent to a ground element of the NRTF-Republic. A single control site is located more than 28 miles from the nearest antenna element. The antenna and control sites each consist of overstory tree plots (existing pole-size stands), plots planted with red pine, and plots of herbaceous plants. The grounding treatment site consists of plots planted with red pine only. No tree stands or herbaceous plots were established at the ELF System grounds because the buffer strips required to eliminate "edge effects" would have placed the study flora at too great a distance from the grounding elements for meaningful EM exposure. Since successive measurements are made on the same plots and individuals without rerandomization over a long period, the experimental design must account for time. Therefore, a combined analysis is made to determine both the average treatment response (site differences) over all years, and the consistency of such responses from year to year, particularly preoperational/operational comparisons.

Tree Growth. Studies have shown that tree growth is sensitive to environmental perturbations other than EM exposure. In order to monitor for possible similar effects from operation of the ELF System, tree growth on existing hardwood stands and pine plantations is being monitored.

As one of the most accepted tree growth measurements, diameter growth is being measured for abundant tree species in the ELF System area (maple, oak, birch, and aspen). Permanently installed dendrometer bands provide continual measurement of diameter growth for each tree in the stand. Other parameters related to hardwood tree productivity such as stand structure, ingrowth, disease, and mortality are used in data analyses; however, these parameters are not specifically addressed in this summary. The magnitude and pattern of diameter increment changes were examined for each species in several ways. Split-plot analysis of covariance was used to compare diameter changes. Models were used to quantify the relationships between tree, site, climate, and tree diameter growth variables. Residual variance and expected growth pattern generated from the model were then used in further analyses.

Except as reported for oak in the investigators' 1990 annual report, ANCOVA showed no significant differences between sites or years (1985-1990) in the growth of the four tree species. Earlier significant differences between years for oak, were not related to energization of the NRTF-Republic. Several of the natural factors used in the statistical analyses continued to show a correlation with EM field intensities, however. A critical requirement in ANCOVA is that the covariates, or natural factors, be independent of the treatments or EM field intensities.

Since there is no known physical basis for the observed relationship, analyses of covariance were used; however, acceptance of the results should be deferred until additional data are collected. The observed relationships may be suprious, and would be verified if future trends diminish.

A diameter growth model was also developed for each species to overcome some limitations in the analysis of covariance. Possible ELF field effects are examined by determining if the differences between the observed and predicted diameter growth values are increasing or decreasing. In the analysis of the residuals, there is no indication that ELF fields are affecting the diameter growth of northern red oak or red maple. For both paper birch and aspen, there were significant correlations between the diameter growth model residuals and the 76 Hz magnetic field. Such correlations were also observed for aspen in 1988. The difference between the antenna and control site in average diameter growth model residuals was consistent with observed differences in previous years for paper birch. For aspen, there has been an increasing difference in average diameter growth model residual between the antenna and control sites with each year of the study.

In summary, there is no evidence in any of the comparisons conducted in the hardwood growth analyses which indicates an effect of ELF fields on the growth of northern red oak or red maple. There is some evidence of an effect of ELF fields on the total annual growth of aspen and, to a lesser extent, paper birch at the antenna site. There is no conclusive evidence of such an effect, however. For example, there were no significant differences in total annual diameter growth for any of the four species indicated in the analysis of covariance. The covariates were correlated with ELF field exposure levels, and therefore confuse interpretation of results. These associations between covariates and ELF fields should not yet be considered reliable in evaluating the effects of the ELF fields on total annual diameter growth.

Young trees experience more rapid rates of growth than older trees; therefore, in addition to monitoring older trees, red pine seedlings were examined for possible growth effects due to exposure to ELF EM fields. After their planting in 1984, pine seedlings at each site were randomly selected for survival and growth studies. For these analyses, each of the marked seedlings was measured at the end of their growing season (1984-1990) for basal diameter and total height. Information on microsite variables, physical damage, terminal bud length, presence of multiple leaders, and number of neighboring seedlings was also collected to aid in interpretations of the statistical analyses. In order to describe the growth pattern, a subsample of seedlings was selected from the marked group for weekly height growth measurements. As for the hardwood trees, total annual height and diameter were analyzed through a split-plot analysis of covariance, while the pattern of height growth within a season was examined through the use of a height growth model.

ANCOVA of total height growth of red pine showed significant differences between years (1986-1989) and site-year interactions. There were no significant differences between sites in height growth. Prior to 1989, height growth was greater for seedlings at the control site than for seedlings at the antenna site. In 1989, height growth was greater at the antenna site. The previous year's air temperature, water-holding capacity of the soil, and soil nitrogen were used as covariates in the analyses. Water-holding capacity has been significantly correlated to one or more of the EM fields at the treatment sites over the past two years. Therefore, the results of the covariate analysis were further examined using models. Significant differences among sites and between years have been evident since 1985. Models based on incremental growth of the leading shoot of the red pine tree were used to evaluate patterns of seasonal height growth. No significant differences between observed and predicted height growth patterns were found for the model's residuals over the period 1986-1989.

There were significant differences between sites and between years (1985-1989), as well as significant site year interaction in the diameter growth of red pine. As in the previous analyses, some of the covariates were correlated with EM exposures. Further examination using multiple range tests showed no consistent pattern of differences between the sites. Correlation analyses indicated a weak, but significant, relationship between magnetic flux density and total seasonal diameter growth only at the antenna site.

Mortality of pine due to *Armillaria* root disease, first documented in 1986, continued during 1990. The root disease is prevalent in many parts of the Great Lakes states. As seedling vulnerability is increased by stressors, EM exposure is being examined as a possible factor in the incidence of pine mortality. At present, this disease is the only natural source of mortality to the pine seedlings. Except for 1989, the frequency of annual mortality increased from 1986 through 1990. In order to make proper statistical comparisons between the sites, pertinent covariates are being identified and measured. These include identification of *Armillaria* clones, numbers and basal areas of hardwood stumps, rock content of soils, mean seedling height, and mean terminal bud length. Additionally, aspen bait stakes have been placed on each plantation in order to obtain additional samples of *Annillaria* for further analysis.

Herbaceous Plants. Like the trees, herbaceous plants are sensitive to environmental perturbations and are an important component of the habitats found near the ELF Communications System. Possible effects to these short-lived plants are being monitored, and the results of ongoing studies are presented in this section. Possible effects to other (long-lived) species (i.e., trees) are presented in the paragraphs dealing with tree growth, above, and litter production, below.

The starflower, an abundant herb in the ELF Communications System area, has been selected for monitoring. Morphological characteristics, as well as select phenological events, of naturally growing starflower plants are being followed each year on plots at the antenna and control sites. Timing aspects related to stem expansion, leaf expansion, and the onset of flowering are the main phenological events being examined. Morphological characteristics being monitored include number of buds, number of flowers, number of fruit, and maximal leaf area.

ANCOVA showed no significant differences between sites in stem expansion (cm/time period), leaf expansion (cm/time period), or leaf area expansion (cm^2/time period). Some differences in the timing of phenological events between sites were observed. Similar analyses showed significant differences between years (1985-1990) for stem length and leaf area expansions. There were no significant interactions between sites and years for these three variables. The covariates used included solar radiation, soil temperature, air temperature, and basal area of adjacent trees (shading). Other climatic factors used as covariates failed to explain any of the significant differences between years.

The proportion of plants flowering at the control site was significantly lower both in 1989 and 1990 than in previous years. Additionally, flowering and fruiting at the antenna site commenced sooner than expected in 1990. As noted above, the timing of some phenological events (e.g., stem expansion, leaf yellowing) occurred earlier at the antenna site, but these trends also were observed in previous years. It is apparent that there are differences in relationships of phenological events between the antenna and control sites, but those relationships remain unknown.

Morphological characteristics of the starflower monitored included number of buds, flowers, and fruit per plant. Other differences in morphological variables were noted. Since similar relationships were seen in previous years, the plants were examined for possible clonal differences between the populations of starflowers at the study sites both in 1989 and again in 1990. Plants and soils were collected at both sites and placed in the same light and temperature regime in a greenhouse at Michigan Technological University (MTU) in 1989. A significantly greater mean number of leaves and leaf width was found on those plants collected from the antenna site. During 1990, randomly-selected plants were selected and interchanged between the antenna site and the control site. The plants transplanted from the control to the antenna site exhibited reduced stem length. No other morphological changes were noted. There is no explanation for the observed differences at this time.

Leaf area was also analyzed by destructive measurement of plants leaves collected at each site over the period (1986-1990). Using regression analyses, linear equations were fit to observations of leaf area; coefficients (slope and intercepts) were then examined for differences between sites and years. Despite significant differences between years (1986-1990), there were no significant differences between sites nor any significant site-year interactions.

To date, the differences detected in intersite patterns of phenological events and morphological characteristics of herbaceous plants cannot be attributed to exposure to EM fields generated by the NRTF-Republic.

Mycorrhizal Populations and Root Growth. Mycorrhizal fungi form a symbiotic relationship with the roots of higher plants such as trees. The fungi utilize organic compounds synthesized by the tree for their growth and to "forage" for minerals and water in the soil. In turn, the fungi provide the tree with minerals and water more efficiently than the tree roots alone. This relationship is considered essential to the satisfactory growth of nearly all tree species. Because the growth of fungal mycelia is dependent on physiologically produced intracellular electrical currents, other sources of electrical current, such as the ELF Communications System, may have an effect on the fungi and, indirectly, on trees. The population dynamics of mycorrhizae occurring on pine trees are being examined.

Populations are being characterized by the frequency of occurrence of mycorrhizal types and the number of mycorrhizal root tips per gram of red pine seedling. As in previous years, Type 3 mycorrhizae were the most common, Type 5 the second most common, and

Type 6 the least common encountered at the study sites in 1990. The total number of mycorrhizae has shown a relatively steady decline over the last several years, including 1990. This interyear pattern is relatable to temperature and precipitation, and/or to seedling age.

Multiple analyses of variance and covariance of all data collected over the period 1985-1990 show no significant differences between sites or interaction of sites and years in the number of mycorrhizae per unit weight of red pine seedling root. Both analyses showed significant differences between years (1985-1990). Detection limits calculated from 1985-1987 data indicate that a dissimilarity of at least 10 to 15 percent will be necessary to recognize a significant difference between sites. An overall disparity of 15 to 25 percent will be required to identify a significant difference between years by site. Findings to date fail to show any measurable effects on mycorrhizal symbiosis between pine and fungi exposed to ELF EM fields.

Foliar Nutrients and Litter Production. The purpose of this element is to examine the nutrient content of growing foliage, the total weight of litter produced, and the nutrient content of (three) litter components. The former monitors for possible timing and magnitude of differences, while the latter two components provide estimates of seasonal canopy production and nutrient inputs to the decomposition system (see Section 2.2).

Actively photosynthesizing red oak foliage was examined for its content of nitrogen, phosphorus, potassium, calcium, and magnesium. Covariate analysis of data collected over the period 1985-1989 showed significant differences between sites in the potassium content of oak foliage and significant differences between years for all five nutrients. Covariates included air temperature, soil temperature, and soil nutrients. Minimum detection levels for differences in the nutrient content of red oak foliage ranged generally from about 10 percent for yearly differences, and over 20 percent for site differences. Multiple range tests showed that in all cases, significant year and site differences occurred prior to intermittent energization of the NRTF-Republic.

ANCOVA of pine foliage nutrients showed no significant differences between sites, but significant differences between years, for all nutrients. Mycorrhizae per gram of root weight, soil nutrients, and climatic variables were used as covariates in the statistical analyses. Many of the covariates proved to be significantly correlated to EM exposures.

Litter was collected in traps on existing hardwood stands at the antenna and control sites. The litter was dried, sorted, and weighed according to the following components: foliage, wood, and miscellaneous. The weight of the foliage component was lower in 1990 than in previous years. A subsample was taken to determine the nutrient content of the litter.

Analysis of covariance showed no significant differences between sites or site-year interactions (1985-1990) for the total weights of the three litter components examined. There was a significant difference between years in the total weight of the miscellaneous category, but no significant differences between years or site-year interactions for the weight of the wood or foliage. Soil and air temperature were used as covariates. The detection limits for interyear and intersite differences have been reduced with the inclusion of 1990 data. Total foliage weights have proven to be more sensitive than the wood or miscellaneous components of the litter.

The total amount of nutrients returned to the soil depends on the nutrient content as well as the weight of the litter. Analyses of covariance showed no significant differences between sites in the nutrient content of litter components or tree species, but several differences between years in the nutrient content of all litter and species categories. Covariates used in the analyses included air temperature, soil temperature, and soil nutrients. Minimum detection levels for litter nutrient content by litter component ranged from 2 to 24 percent, and by tree species from 2 to 25 percent of the mean. Significant differences were further examined to determine if nutrient content had changed in response to NRTF-Republic operation. Multiple range tests using covariate adjusted means showed some of the differences between sites existed before initiation of intermittent operation at this facility.

Results to date indicate that operation of the facility to date has had no detectable effects on the nutrient content of actively photosynthesizing foliage, production of tree litter, or the nutrient content of litter.

2.2 SOIL MICROFLORA

Soil microflora (bacteria and fungi) play a key role in the maintenance of upland forest ecosystems such as those in the ELF Communications System area. They degrade organic molecules present in litter and influence the size of other microbial populations that have important influences on the nutrition of plants. Anthropogenic factors that disrupt soil community processes may directly alter the flow of nutrients to vegetation and thus indirectly affect the forest community. The objectives of this element are to monitor for possible effects from EM fields produced by the ELF Communications System on populations of streptomycete bacteria associated with plant roots and to examine overall rates of litter decomposition.

Upland flora (producers) and soil microflora (decomposers) form a natural assemblage. Although these elements are being examined as separate projects, both subcontractors are with the Department of Forestry, Michigan Technological University, and both share common study sites and ambient monitoring systems. Bacterial population and decomposition objectives are closely related to the mycorrhizal and litter production objectives described in Section 2.1. Studies of other important soil organisms can be found in Sections 2.3 and 2.4.

Streptomycete Bacteria. Streptomycetes have been reported to be involved in the nutrition of mycorrhizae and may indirectly influence trees through their production of antibiotics or growth factors. The purpose of this element is to characterize and enumerate streptomycete bacteria associated with red pine mycorrhizae (see Section 2.1).

Samples were taken monthly from May through October from pine trees located on plantations at the antenna, ground, and control sites. Sample sizes and protocols used during 1990 were the same as used in previous years. Macerate plate count data for morphotypes and population levels associated with Type 3 mycorrhizal fine roots were expressed as numbers per gram of root. Samples were also analyzed for bacterial-isolate ability to degrade important organic molecules. All data were logarithmically transformed prior to statistical analyses. Two-way analysis of variance was used to compare sampling dates and study sites within 1990. Three-way analysis of variance was used to compare years (1985-1990), as well as sites and sampling dates. Whenever these analyses showed significant differences, multiple comparisons were conducted by least square mean procedure to determine the relationships of the variables. Covariates, particularly weather-related parameters, were also included to further examine differences between sites, years, or sampling dates.

As has been the case since 1985, Type 3 mycorrhizae continued to predominate in 1990 at antenna, ground, and control red pine seedling plantations. Total numbers of mycorrhizae per gram of root were slightly higher than in 1988 and 1989, and remained lower than for 1985-1987. No significant differences were observed between data sets.

The streptomycete level and morphotype data for 1990 were compared with data for 1985-1989. The degradative capacities of morphotypes have been constant since 1985. Two-way ANOVA was used to detect significant differences between data obtained in 1990 at each plantation; three-way ANOVA was used for yearly comparisons, monthly comparisons, and comparisons between plantations. ANCOVA was also used in 1990 to interpret differences. To date, soil temperature at a depth of 5 cm and precipitation factors appear to be important.

The 1990 results can be summarized as follows: There generally were no significant differences in streptomycete levels or morphotype numbers. As in past years, the detected significant differences were found between yearly and monthly data. However, streptomycete levels at control sites were found to be lower in 1990 than at antenna and ground sites. In general, morphotype B was detected at all sites, and was often the predominant type. ANCOVA appears to adequately identify factors that contribute to observed differences, and those factors appear to be independent of EM field exposure.

Litter Decomposition. Mass loss of leaf litter is a sensitive index of organic matter deterioration and has traditionally been a measure of the overall functioning of the litter community. This study element monitors the decomposition of leaf litter from three species of trees found in the ELF Communications System area. The species are northern red oak and red maple, which are common, and red pine, which are found as scattered specimens throughout the area.

Leaf litter is collected each autumn from a single location. The leaves are either archived for possible future reference and nutrient content analysis, or weighed and enclosed in nylon mesh envelopes for emplacement at study sites. The envelopes emplaced at study sites contain either individual leaves or bulk foliage samples of a single species. All samples are emplaced in December. A few samples are retrieved each month over the period April through November of the following year. Retrieved samples are reweighed, and data are expressed as the percentage of original dry matter mass remaining. Mass loss data from 1985 through 1990 are complete.

After arcsin square root transformation, litter mass loss data were examined by ANOVA and ANCOVA. Prior to 1990, ANOVA and ANCOVA were conducted by means of a linear effects model. Commencing in 1990, a means model has also been used in the ANOVA analyses. The models are mathematically equivalent, but the latter permits more accurately examined temporal trends at the study sites.

The observations from ANOVA of data obtained since 1985 can be summarized as follows:

- decomposition of oak leaf and maple leaf litter emplaced in hardwood stands and pine plantations was not significantly different at the antenna, ground, and control sites;
- decomposition of pine needles emplaced in hardwood stands occurred faster at the antenna site than at the other sites; however, decomposition of pine needles emplaced in pine plantations occurred fastest at the ground and control sites;
- during 1990, oak leaves emplaced in hardwood stands decomposed faster than in previous years, and those emplaced in pine plantations decomposed nearly as fast as in 1989 (the fastest year to date);
- decomposition of maple leaves in both hardwood stands and pine plantations was not quite as slow in 1990 as in 1989 (the slowest year to date);
- pine needle decomposition for samples emplaced in both hardwood stands and pine plantations was slower in 1990 than in any other year since 1985.

The ANOVA further demonstrated that the decomposition of leaf and needle litter in hardwood stands has been consistently faster at the antenna sites than at the ground and control sites from 1988 through 1990. No significant differences had been detected prior to 1988.

Year-to-year trends of litter decomposition in pine plantations were inconsistent among the antenna, intermediate, and control sites in earlier years. Since 1988, however, no significant differences have been detected among the plantations.

ANCOVA is used to identify factions that appear to influence litter decomposition. Five classes of covariates are examined in the litter studies: annual litter collections prior to dispersal (e.g., initial lignin content), individual samples prior to dispersal, (e.g., individual oak leaf density), retrieved samples (e.g., nitrogen and phosphorus content), unchanging temporal aspects (e.g., distribution of stumps), and dynamic aspects of sites (e.g., weather factors, density, and height of pine seedlings). As noted above, EM field exposure had been omitted from covariate analyses prior to 1990, and has been included in 1990 analyses.

The inclusion of EM field exposure in ANCOVA raises several statistical concerns that remain to be resolved. For example, it is not known whether relationships between EM field exposure and litter decomposition, if suggested by ANCOVA, are real or spurious. No hypothesis for such a relationship exists, and a fundamental precept of this project is that existing hypotheses are tested, or new hypotheses eventually may be developed from project analyses.

ANCOVA has proven useful in explaining most, but not all, of the statistically significant differences in decomposition detected by ANOVA since 1986. The inclusion of 60 Hz and 76 Hz EM field data has improved the interpretation of results, and is a major finding of the 1990 analyses. However, several years of additional data are needed to either refute or support the proposition that EM field exposure may be a contributing factor in observed variations in leaf litter decomposition.

2.3 SOIL AMOEBAE

Soil amoebae are common soil organisms that are predators on bacteria. Bacteria, in turn, are important to the soil ecosystem because of their ability to mobilize nutrients needed for plant growth. To the extent that protozoa affect the number and types of bacteria in the soil, they also become a potentially important factor in soil fertility. Studies on protozoa and other closely related organisms have suggested possible EM effects on characteristics such as orientation, growth, and physiology.

In order to examine for possible effects from the operation of the ELF Communications System, the following aspects of soil amoebae are being studied:

- species and strain characteristics
- population size and activity
- growth and feeding

In addition, selected elements indicative of soil fertility are being monitored.

Studies on soil amoebae are being performed at three study sites in Michigan. One treatment site is located adjacent to an overhead antenna wire of the NRTF-Republic; the other is located adjacent to a buried grounding element of that facility. A third site, the control, is located about nine miles from the nearest ELF System element.

Species and Strain Characterization. During 1990, as in previous years, eight types of amoebae (various generic and species levels) were isolated using soil enrichment techniques. To date, no important differences between years or between sites have been reported in the types of amoebae present.

The genetic diversity within a single species of soil amoeba, *Acanthamoeba polyphaga*, had been determined by isoenzyme analysis, but budget constraints have prohibited continuing this aspect of the study. Analyses of data through 1988 showed no significant differences between sites in the genetic diversity of strains of *A. polyphaga*. The genetic diversity of this species apparently decreased over the period 1985-1988, probably because of adverse precipitation conditions.

Population Size and Activity. The size of the amoeba population is considered an ecological variable likely to influence the functioning of the soil system.

Soil samples for population studies were taken with a coring device. Coring locations within study sites were determined randomly, using a numbered grid system and a random number generator. The soil profile at study sites is typical of northern hardwood soils--i.e., with a sharp difference between the upper, organic horizon and the lower, mineral horizon. In a typical core, the 1- to 2-in. organic horizon is taken as one sample, and the top 2 in. of the underlying mineral horizon is taken as a second sample. A soil-dilution counting technique is used to determine the population size of each sample.

Studies to date have shown that the total amoeba population at any given moment consists of both vegetative (actively reproducing) and encysted forms. During both the 1984 and 1985 growing seasons, marked cyclic changes occurred in the total number of amoebae present; the number of vegetative stages and total amoebae often increased or decreased by two orders of magnitude over short periods of time. Since 1986, the occurrence of fluctuations and the overall number of amoebae have been relatively small.

ANOVA of 1989 data showed a significant difference between sites in the total number of amoebae and the total number of cysts present in the organic horizon of the soil during July. The number of vegetative stages increased at the antenna site, but not at the other sites.

The 1990 data also showed some differences in total population size among sites. Unlike 1989, however, the population was higher at the control site, and the difference occurred in September. The differences again occurred in the organic horizon of the soil.

__**Growth and Feeding Activity**__. The purpose of this element is to determine the *in situ* growth and feeding activity (i.e., predation on bacteria) of soil amoebae in buried culture chambers.

This study element involves suspending a known species of amoeba (*A. polyphaga*) and a food bacterium in a physiological saline, all contained in a culture chamber. In order to simulate electric fields and currents present in the surrounding soil, the chambers are connected to buried collecting electrodes. Culture media with bacteria are replaced on a two- to three-week cycle using EM-exposed amoebae from old cultures to inoculate the new media. Periodic counts of amoebae were made to determine changes in the number of organisms. A logarithmic transform of the growth data provided a straight-line plot (numbers over time), which was then quantified by regression analysis. Using a modified t-test, the resulting slopes of the lines were compared to examine for statistically significant differences between sites.

In 1990, as had been the case in previous years, there were no significant differences between sites in the growth, or for the isoenzyme heterogeneity, of the cultured amoebae. The 1990 findings are especially important because improved culture chambers were used and data analysis improvements were made.

2.4 SOIL AND LITTER ARTHROPODS AND EARTHWORMS

Arthropods and earthworms play a major role in the decomposition of vegetation. These invertebrates shred plant material such as leaves and redistribute the remains in the soil habitat. The vegetative remains are then further degraded by soil microflora (see Section 2.2). For the purpose of detecting possible effects of the ELF Communications System on major agents of litter decomposition, this project is monitoring both the structural and functional aspects of the litter and soil invertebrate community.

The project uses one treatment site located adjacent to the antenna ROW at the NRTF-Republic and one control site located at a distance west of the antenna. Both sites are situated in a maple-dominated deciduous forest. Although the sites display faunal differences, they have similar soils, vegetation, and microclimate.

In order to address faunal differences between sites, community indices and the characteristics of major populations common to both sites are emphasized in these studies.

In addition to dominant groups, populations representing various roles in the soil habitat, such as predators and detritivores, are examined. To accommodate the various roles of the soil fauna, intersite comparisons of ecological equivalents and/or preoperational and operational comparisons of populations unique to the treatment site are planned. Litter decomposition rates will provide an overall indication of the functional aspects of the soil community.

Surface-Active Arthropods. This element examines the major arthropod fauna using the surface layers of the soil at each site.

Diel and seasonal activity patterns of surface-active arthropods were assessed by consecutive, day-and-night, pit-trap samples taken once a week. In order to increase catches of surface-active arthropods, pit traps were provided with barriers that increased the effective area sampled by diverting moving arthropods toward the pit. Major groups trapped were springtails, mites, and ground beetles.

Since 1985, 36 species of springtails have been identified, with about 75-85 percent of the species shared between sites. Marked differences prevail between sites and between years in the diversity of the springtail community. Based on the data collected and analyzed to date (through 1989), the sites also differed in the relative dominance of major species as well as in the occurrence of a few rare species. There were large differences between years and sites in the total number of springtails trapped. It should be noted that data analysis lags behind data collection by one year.

Monitoring of the activity patterns and density of three abundant species of mites continued during 1990. Generally, intersite seasonal activity patterns remain highly correlated for all three species.

Since 1985, 24 species of ground beetles have been identified. The number of species has remained relatively constant, and is similar between sites. Preliminary examination, by means of linear regression statistical techniques, of the pattern of numbers trapped throughout the season showed no significant differences between sites within a given year. However, the total numbers trapped at the control site in 1988 and 1989 were markedly lower than those trapped at the treatment site. In addition to monitoring diversity and trappable numbers (activity pattern), researchers are examining fecundity (number of eggs per female) as an indicator of the physiological state of adults.

Four species of beetles are studied with respect to fecundity. Analysis of data collected over four years (1985-1988) was completed during 1990. In addition to fecundity, sex ratios have been determined, and developmental patterns were identified in considerable detail for the first time. No significant differences between sites have been observed in fecundity among the four species on a year-by-year basis. Comparisons within years appear to be most meaningful because numbers of captured females vary greatly from year to year.

Activity cycles had been found to be well-correlated between sites during past years, and the correlation held in analysis of 1989 data as well. Five distinguishable stages of the female cycle have been identified, and the temporal patterns of the stages for several species are now understood. This understanding is expected to be an important basis for evidence of potential disturbance in future years.

Soil and Litter Arthropods. The population and community dynamics of soil and litter arthropods are being determined from samples taken during the growing season (May-October). Litter and soil are sampled separately. The arthropods are then extracted by heat and sugar flotation techniques. Springtails and mites are the most numerous taxa in the litter and soil of both sites and are the major groups of interest. At the time of reporting, data were available for the period 1984-1989.

Since 1984, 73 species of springtails have been identified at the two study sites, and in any given year 45-55 species have been collected. The diversity of the springtail communities shows some differences between sites and years, but since 1986 diversity indices have declined in a similar manner at both sites. Diversity indices appear to have stabilized in 1989, the latest year for which analyses have been completed. Temporal fluctuations in total numbers and developmental stages are highly correlated between sites. Several species continue to occur in large numbers at both sites. The most abundant species with high correlations in intersite densities have been selected for intensive characterization. There have been no significant differences through 1989 between sites or years in the mean number of individuals constituting three developmental stages (hatchlings, juveniles, or adults).

Identification and enumeration of all mite species found in the litter and soil is an intractable problem; therefore, three relatively abundant species have been selected for this monitoring effort. Year-to-year changes in density are similar at both sites for each of the three species. Two of the three species also showed a highly correlated population composition (density of larvae, protonymphs, deutonymphs, and adults) between the study sites. Monthly frequencies of developmental stages of two species were analyzed in detail using ANOVA. Both species of mites showed the same results: significant differences between years but not between sites. The absence of significant site-year interactions further indicated that between-year differences occurred in parallel at both the treatment and control sites.

Earthworms. The purpose of this element is to examine the major earthworm fauna inhabiting the soil and litter of the study sites.

Earthworms were extracted from litter using weak formalin, and those in soil were obtained by hand sorting followed by wet sieving. Earthworm samples were taken at regular intervals from May through October. Nine species of earthworms have been identified at the study sites. As expected, species diversity indices are low but are comparable to those of worm communities found in similar latitudes and habitats. Eight of the nine species are found at both the treatment and control sites, but their densities are markedly different between sites.

Both 1989 and 1990 are considered to be ELF operational years for purposes of analyzing earthworm data. Based on comparisons of 1989/1990 observations and predictions, it has been concluded that several of the most common species at the test site have not appeared in the abundance that should be expected. Temperature and soil moisture conditions of one or two previous years (depending on species) would indicate that numbers should have been higher in several age brackets.

It is not known at this time if electric and/or magnetic fields produced in soil by the ELF Communications System, some other environmental parameters, or the predictive technique itself might be associated with differences between predicted and observed results. Several more years of ELF operations experience are necessary (because of earthworm life cycle and generational patterns) to detect an EM field effect. Special analytical methods and experiments are required to further examine the earthworm densities at the antenna sites.

Litter Decomposition. Litter decomposition provides an estimate of the overall functioning of all soil biota involved in organic matter breakdown and nutrient release. This system-level response complements the faunal parameters under investigation and provides a context to evaluate effects seen at the populational and organismal levels of organization.

Estimates of litter decay rates were obtained by examination of mass loss from leaves of known initial weight. Samples of dried maple litter were weighed and placed in mesh netting on the soil surface at both study sites. At intervals throughout the year, samples were retrieved, dried, and weighed. Correction for soil contamination was determined by combusting ground samples and weighing the residue. Analysis of samples taken over the period from May 1986 through September 1987 showed no significant differences between sites in litter mass loss. A litterbag series implemented in November 1988, and sampled throughout 1989, showed significantly greater mass loss at the treatment site than at the control site for October and November periods. There were no significant differences between years at the treatment site; however, decomposition was greater in 1986 than in 1989 at the control site. It was presumed in 1989 that the near-absence of one consumer species might be the reason for the observed result.

Litter inputs were determined by collection of leaves in litter traps located at each site. Traps were emptied weekly during the time of greatest leaf fall and monthly at other times. Samples were sorted by category, then oven-dried, cooled, and weighed. In 1989, total litterfall (g/m^2) was insignificantly larger at the treatment site than that at the control site. Previously, total litterfall was significantly greater at the control site. Input values at both sites continue to be consistent with data reported for forests at similar latitudes.

Previous analyses of the amounts of litter on the forest floor (standing crop) showed no significant difference between sites in October, the time of the maximum. The standing crop of litter, however, was significantly higher on the control site during most of the remaining season. In order to improve these estimates, ashed dry weight was determined for available samples (1987-1989). ANOVA showed that for dry weight estimates, site differences were not significant, nor were there site/date or site/date/year interactions. These results indicate similar patterns of input and decomposition at both sites. Significant within-site variability was found, and significant differences were also found between years.

The 1990 observations were similar to observations in previous years. The standing crop available for decomposition was larger at the control site than at the test site. Additionally, decomposition occurred at a slightly lower rate at the former than at the latter. The turnover rate is nominally about one year at each site.

Although the decomposition rate was lower at the control site during 1990, the rate was not as slow as in 1989. It was observed that the consumer species that was low in 1989 appeared to be making a comeback in 1990. This observation tends to add credence to the 1989 proposition that the species, and not the initiation of ELF testing, was the likely reason for the observed results.

2.5 NATIVE BEES

Enervated cells containing iron granules have been found in the abdominal segments of foraging honeybees. It has been speculated that these iron structures may play a role in orientation and may provide a basis for the sensing of EM fields by bees. Behavioral changes such as increased dispersal, increased levels of activity, lowered overwintering survival, and modification of nest structure have been described as effects from fluctuations in the earth's magnetic field and from exposure to the EM environment associated with transmission lines.

Honeybees are rare in the forested areas in which the ELF Communications System is located. Native bees, however, are abundant and are of particular importance to ecological communities in the area as pollinators of the resident flowering plants. Native bees have coevolved with resident plants and are able to overwinter in the study area. For these reasons, native bees, rather than honeybees, are being studied. Aspects of nesting activity, nest architecture, and the mortality of native bees have been monitored for possible EM effects from the operation of the ELF Communications System.

Observations on native bees have been made at two treatment sites and two control sites since 1983. Data on nesting activity were collected by direct observation as bees were constructing their nests. Information on nest architecture and mortality were collected using trap nest techniques, which involve setting predrilled blocks of wood on shelved hutches and allowing bees to construct nests.

Each nest consists of a series of reproductive (cell) and nonreproductive (basal and vestibular) spaces within the bore of the hole. Each cell is lined with elongate leaves and is provisioned with pollen. After an egg is deposited, the open end of the cell is closed by a partition consisting of rounded leaves. The ends of the nonreproductive spaces are also closed with a series of plugs consisting of rounded leaves and other material. Generally, the egg hatches and the larva molts through a series of stages to overwinter as a prepupa.

More than 40 species of native bees are known to occur in the ELF Communications System area, 20 of which will use trap nests. This study focuses on two abundant species, *Megachile inermis* and *M. relativa*.

<u>Nesting Activity</u>. Disorientation and agitation have been reported for honeybees foraging or building nests near transmission lines. This element examines for similar behaviors by observing the duration of foraging trips made by native bees.

From 1983 through 1986, an extensive effort was put forth in observing, recording, and determining the activity patterns of various species of native bees. Analyses showed that the duration of trips for nest material to cap cells (round leaves) was relatively short, and less variable than other foraging behaviors, such as pollen collection. Because *M. inermis* was relatively more active and easier to identify than other species, its leaf foraging behavior was selected for further study as a possible indicator of disorientation or agitation.

From 1983 through 1990, the foraging time of bees on the control site has been consistently shorter than that of bees located on the treatment site. In order to account for trip rank and environmental factors, data collected since 1987 were extensively examined using a general linear model for ANOVA. Based on these analyses of the 1987-1990 data, the antenna is not affecting trip durations. In addition, the difference between foraging times for bees located at treatment and control sites has been less than the minimum difference detectable. Data collected prior to 1987 will be reanalyzed during 1991 using the general linear model.

<u>Nest Architecture and Orientation</u>. When honeybees were exposed to EM fields produced by high-voltage transmission lines, their reproductive output was lowered, and they increased the amount of propolis at their nest entrance. Other reports indicate that under certain conditions honeybees may use the earth's magnetic field to orient their comb. If native bees respond to the EM fields produced by the ELF Communications System, they may alter architectural aspects of their nests in such a manner as to become less competitive than bees exposed to markedly lower EM intensities. In order to examine for this possibility, researchers are monitoring the size, number, linings, cap thickness, and orientation of cells produced by *M. inermis* and/or *M. relativa*.

In all years through 1989, *M. relativa* produced similar numbers of nests at all sites. (1990 data are not included here because nests will not be examined until 1991; that is, after overwintering of the 1990 nests.) The few observed significant differences were not consistent between sites. The other species of interest, *M. inermis*, has consistently produced fewer nests at the control sites than at the treatment sites. Vegetation differences appear to account for the differences in numbers of nests, and therefore there is no suggestion that ELF EM fields are involved in the discrepancies. Moreover, the differences were recorded even before operations commenced at the NRTF-Republic.

In addition to counting numbers of nests at each study site each year, the lengths of cells in each nest are determined. No consistent differences in cell length have been found between treatment and control sites for *M. relativa*, either prior to or subsequent to commencing ELF System operations. Longer cells have been consistently found at the control sites for *M. inermis*. The consistency has held since ELF operations commenced.

A third nest parameter of interest in this study is the number of cells per nest each year. Over the years, substantial variations in cells per nest have been found for *M. relativa*, and somewhat less variability has been observed for *M. inermis*. Ordinary environmental changes (e.g., precipitation) explained much of the variability, and to date there is no suggestion that EM field exposure is responsible for the variability. The number of leaves used by both species to line their nests does not appear to be affected by the operation of the NRTF-Republic. Neither significant site differences nor intrinsic trends have been observed for this parameter.

It has been suggested by other investigations that the orientation of beehives with respect to the earth's magnetic field may be important. Although it is not known whether orientation dependency is important to megachilid bees, orientation is being examined as a part of this study. Some nests are intentionally oriented in the north-south direction at each site, and others are oriented in the east-west direction. Additionally, some nests are moved seasonally between treatment and control sites. Only the nests of one species (*M. relativa*) are being studied in this regard, and analysis of data has been completed through the 1988 season.

A directional bias has been found for some sets of nests, but the directional bias has not been the same for all sets. Thus far, there is no evidence that directional bias, where it has been observed to exist, has been influenced by ELF System operations.

Emergence and Mortality. High-voltage transmission lines have been reported to lower the overwintering survival of honeybee colonies. In order to monitor for a possible similar effect on native bees, researchers are examining the proportion of nest cells that produce adults and the sources of mortality at treatment and control sites.

Completed nests were allowed to overwinter at study sites. During the spring, the nests were removed from the sites and taken to a laboratory, where they were split open. After data on nest architecture were recorded, cells were placed in individual plastic culture tubes. Tubes and cells were then kept outdoors at ambient temperature until emergence. Date of emergence, species, and sex of offspring were then recorded. Adults were released at the sites where their nest had been constructed the previous summer. Cells that showed no signs of emergence were opened, and the contents were examined to determine the condition of the bee.

Prior to their emergence in late spring, native bees are subject to mortality during any of several developmental stages (egg, larva, prepupa, pupa, or adult). Failure to emerge is used as an indication of morbidity, and the time of occurrence is associated with the developmental stage. Pre-overwintering mortality is related to the egg and larval stages, and overwintering mortality to the prepupal and later stages.

Although it is relatively simple to detect mortality in megachilid bee nests after overwintering, it is much more difficult to determine precisely when mortality might have occurred, and what the causes of mortality might have been. The emergence/mortality portion of this study also has been complicated by an unavoidable change in study protocols. In early years, nests were removed from the field in the fall and placed in a laboratory. In later years, the nests overwintered at their respective field sites, and were brought to the laboratory only briefly to study nest architecture. The location of the laboratory also changed.

These confounding factors limit the ability to analyze mortality and attribute causes of mortality with desired confidence. Some changes in analytical methods were made in 1990 to overcome the confounders. It is now clear that mortality prior to winter was greater in 1988 and 1989 than in previous years. The summer of 1988 was exceptionally hot and dry; the spring of 1989 was unusually cold. Overwintering mortality was not exceptional in either year. The ambient environmental conditions undoubtedly caused much if not all of the pre-winter mortality, and conclusions regarding ELF EM field exposure cannot yet be reached.

2.6 SMALL MAMMALS AND NESTING BIRDS

Some laboratory studies performed at commercial power frequencies and pulsed ELF frequencies have indicated effects on small vertebrates. The applicability of these findings to vertebrates exposed to EM fields from operation of the ELF Communications System is vague. In order to address this concern, important biological and ecological characteristics of small bird and mammal species residing in the ELF System area have been monitored by researchers from Michigan State University (MSU).

Until 1988, both community and populational aspects of resident mammals were monitored. A high variability in results was found, and these studies have been discontinued. However, community and population characteristics of birds have been monitored by investigators from the University of Minnesota-Duluth (UMD) since 1984 (see Section 2.7). This MSU study continues to examine individual aspects of select mammal and bird species, including reproductive, developmental, and physiological characteristics.

Those species selected for studies of most individual attributes are the deer mouse, chipmunk, and tree swallow. The black-capped chickadee is also being examined, but solely for physiological variables. The project uses five treatment sites in, or immediately adjacent to, the antenna ROW and four control sites with habitats similar to those at the treatment sites. Areas at the control sites have been cleared (sham ROWs) and are being managed the same as the antenna ROW.

Embryonic Development. Prenatal developmental stages have been shown to be sensitive to many types of environmental perturbations. Although different from the fields produced by the ELF System (see the introductory paragraph), some EM fields have been reported to affect embryonic development. Indirect effects on development may be possible, should EM exposure affect parenting behavior. Possible EM effects on prenatal development are being monitored using the incidence of abnormalities for embryos of tree swallows nesting near the ELF Communications System. (The prenatal development of mammals is not being studied because of the probable adverse effects on naturally low population levels that would result from the sacrifice of reproductive females.)

Embryos of tree swallows were collected at three treatment and two control study sites after four days of incubation. The embryos were dissected from the egg, preserved, and then examined microscopically. Each egg was coded so that the investigator who examined for abnormalities was unaware of the source site. The following were checked for abnormalities: head, heart, branchial arches, spinal cord and somites, limb buds, allantois, and amnion, as well as the flexion and rotation of the embryo.

Several types of abnormalities have been identified, including lack of development, chaotic development, rotation reversals, spine abnormalities, allantois reversals, and posterior-directed allantois. Normal development was somewhat slower in 1990 than in previous years. Slower development was not surprising, since 1990 temperatures were markedly lower than in earlier years of this study. As had been observed in the past, abnormal development was found in 1990 in embryos taken from treatment sites and control sites. Statistically significant differences in the occurrence of abnormalities were found among all nests, but no significant differences were found between all nests at treatment sites and all nests at control sites. About 20 percent of the embryos examined were abnormal, a figure comparable to data (15 to 20 percent) reported in the literature. The kinds of abnormalities found in this study were not different than those reported in the literature.

Since avian embryos must develop in a closed system, the resources allocated to each offspring during oogenesis could have a marked influence in determining chick survival. To determine whether operation of the ELF System adversely affects the amount of nutrient deposited, each egg was weighed and measured at the time of collection. There were no significant differences in mean weights or volumes of eggs collected during 1990 at treatment and control sites. This result is consistent with findings in earlier years.

Fecundity, Growth, and Maturation: Tree Swallows. The purpose of this element is to monitor important aspects of the reproductive and postnatal growth processes in tree swallows. The variables are numbers of eggs per clutch, hatching success within clutches, rates of postnatal growth, development of hatchlings, and nestling mortality.

Studies are carried out in clearings where arrays of nest boxes have been placed. The boxes can be opened to permit observation and measurement of the young. Active nests are checked daily or every other day to determine the dates that eggs are laid, the number of eggs, hatching dates, and overall hatching success. Monitoring of the nests for nestling growth and mortality then continues until all young fledge. Attempts to monitor parental attentiveness to nestlings using video recording devices or temperature probes were discontinued at the end of the 1988 field season because the results were too variable to permit a structured analysis.

Clutch size (maximum number of eggs laid in a nest) has been used as an indicator of fecundity. As in prior years, there were no significant differences between study sites in 1990 in mean clutch size. Mean clutch size was between 5 and 6 eggs per nest. Analyses of data pooled over the years show no significant differences between sites or years; no plot/year interactions were noted. Researchers continue to collect and analyze data on available food supply (insect biomass) as one possible factor influencing clutch size.

Hatching success during 1990 was between 90 and 95 percent at the treatment and control sites; this slight difference was not statistically significant. According to analysis of data pooled over the period 1985-1990, the likelihood to hatch was independent of both site and year. The actual number of birds to hatch in 1990 was about 5 at both the treatment site and the control site. ANOVA of hatch frequency data over the years shows significant differences between years but not between test and control sites.

No significant difference between sites was found during 1990 in the postnatal growth indicators of mean number of days to eye opening or feather eruption. In 1990, the mean number of days to eye opening was only about 6 at treatment and control sites; the mean number of days to feather eruption was slightly less than 8. Both eye opening and feather eruption occurred sooner in 1990 than in previous years. Data pooled over the years showed significant differences between nests, but none between sites, for these two developmental parameters.

In order to examine growth rates, periodically measured values were fit to models. Data on body weight, tarsus length, and ulna length (1985-1990) best fit logistic models, whereas data on wing length best fit an exponential model. The models were used to produce parameters (e.g., growth rate constants or rate of growth at the inflection point) that were then examined by nested ANOVA. No significant differences between sites were detected for 1990 or previous years, with one exception: the tarsal growth constant was significantly larger ᵢ)89 for birds on the treatment site than for birds on the control site. Tarsal growth constant was not different between sites in 1990.

The likelihood of young tree swallows to fledge has been monitored since 1985. Significant differences have been found in fledging success between years and between sites. The yearly variability has been caused mainly by adverse weather following hatching in 1986 and in 1989. The significant difference between sites would not have occurred were it not for high mortality by natural predation at the control site during 1990. The fledge rate in 1990 was higher at the treatment site than at the control site, a trend that has been quite consistent for the last six years. Fledging success during 1990 at both sites was relatively high (between 3 and 4 birds).

Values obtained in these studies of fledging success, hatching success, and clutch size are similar to those reported in the literature for other studies of tree swallows. The overall mortality of eggs and nestlings has not been significantly different between treatment and control sites.

Fecundity, Growth, and Maturation: Deer Mice. The purpose of this element is to monitor important aspects of the reproductive and growth processes in deer mice. Rates of postnatal growth and development of nestlings were examined during 1990. Previously, maternal attentiveness to nestlings, number of young born per litter, and the proportion of young surviving until weaning were also monitored.

Large, open enclosures were used to restrict the movements of deer mice during studies of postnatal growth and development. The deer mice to be studied were captured in mixed deciduous forests near the enclosure sites. The animals were paired, and when the female was pregnant, she was transferred to the large enclosure to give birth and rear the young to weaning. Observations were then made while the young were located in a nest within the enclosure.

Growth studies to date have shown that growth curves of temporal change in the body mass of nestlings are different between litters. Growth rates, therefore, have been estimated using linear regression analyses for the growth of each individual in a litter. Examination of individual growth during 1989 using ANOVA showed significant differences between litters (a "mothering" effect), but not between sites. In 1990, as in previous years, the age at incisor eruption was not significantly different between sites. Age of eye opening at the test site was earlier than at the control site. Year-to-year eye opening has not been consistent among sites.

Homing Studies. Published information suggests that magnetic fields are one of several cues used in the orientation of some birds and mammals. Since animals are able to find food and escape predators more effectively in their home range, any disturbance of the ability to return to, or use, a home range could decrease an animal's probability of survival. Monitoring of the homing ability of tree swallows and deer mice is in progress, therefore, to assess for possible effects from the operation of the ELF Communications System. The variables being examined are the likelihood to return (number of displaced individuals that return home), and, with tree swallows, the amount of time taken to return home.

Brooding birds were captured at nest boxes, marked, and taken to release sites some 30 km distant. Birds captured at treatment sites had to cross both east-west antenna elements in order to return to their broods. Birds captured at control sites were displaced at angles and distances similar to those used for birds captured at treatment sites, but did not cross or come close to any antenna elements in returning to their nest boxes at the control site. Investigators recorded the time at which the birds returned. Previous studies have shown no significant differences between genders or direction of displacement in the return times of birds captured at the treatment or control sites.

ANOVA showed significant differences between sites and between years in the time taken by birds in returning to their capture site. Further analyses indicated that the course or capture site, and not temporal factors, was responsible for these results. When each year is examined separately, return times to the treatment site were consistently shorter than return times to the control site. For example, in 1990, return time to treatment sites was 148 minutes, while return time to control sites was 172 minutes. There may be intrinsic differences between return courses, as more birds fail to return to control sites than to treatment sites.

A concerted effort was made in 1990 to identify possible reasons for observed homing differences (EM field exposure is not likely to be the cause, since the differences were found in preoperational as well as operational years). Thus far, differences in release points have been tentatively ruled out, and studies will concentrate on characteristics of home sites.

Chipmunks and deer mice were captured on a trapping grid at treatment and control sites. Displacements took place during, or just prior to, the next activity period following capture; deer mice were displaced at dusk and chipmunks in the morning. Individuals were displaced either to the south or west of the trapping grid, with each animal displaced 450 m from the trap at which it was captured. The displacements to the south were through relatively continuous forest, whereas displacements to the west required the returning animals to cross the antenna ROW or sham ROW. Once an animal was displaced, traps on the grid were checked morning and evening for the next five days.

During the preoperational years (1986-1988), few differences in homing success were found for either deer mice or chipmunks. Chipmunk homing during 1989 and 1990 was consistent with data collected in 1986-1988. However, deer mouse homing showed inconsistencies with previous years in both 1989 and 1990. In 1989, deer mice homed more successfully at the treatment plot; in 1990, they homed more successfully at the control site. It is not possible at this time to assess whether ELF operations are a factor in deer mouse homing behavior.

Physiology: Peak Aerobic Metabolism. The purpose of this element is to determine the peak aerobic metabolism of chickadees and deer mice during an annual period of severe stress (winter). This variable provides a general index of an animal's health.

Black-capped chickadees and deer mice were collected during the winter along the ELF Communications System ROW and at a control site. Animals to be tested were held at an outdoor facility, with food and water provided ad libitum. Tests for peak metabolism were performed in an ethanol-cooled chamber, using a version of the helium-oxygen method. Test equipment was located at a laboratory in Crystal Falls, Michigan; the holding facility was situated several miles south of the city. Studies have shown that the peak metabolic rates of deer mice and chickadees do not change during the three weeks in the outdoor cages. After testing, animals were released at their collection site.

Previous analyses of data pooled over the period 1986-1989 indicated no significant differences between sites or between years for deer mouse metabolic rates. Similar analyses showed these rates for birds captured on control sites to be slightly, but significantly, higher (4 percent) than for birds captured on treatment sites. There were no significant differences between years or between year-site interactions. It was concluded that in the years prior to full operation of the NRTF-Republic (1986-1988) peak metabolic rates were stable for deer mice and chickadees from year to year, similar at both treatment and control sites for deer mice, but somewhat different between sites for chickadees. Single-factor ANCOVA showed no significant differences between sites in the peak (weight-specific) metabolic rate for deer mice or chickadees examined during 1989 and 1990, the years of intermittent and full-time operation, respectively, of the ELF Communications System.

2.7 BIRD SPECIES AND COMMUNITIES

Birds are sensitive to magnetic fields and use such cues, along with others, for orientation. To examine for possible adverse effects from EM fields produced by the ELF Communications System, species and community characteristics of birds that migrate and breed in areas adjacent to ELF transmitting facilities have been monitored. Studies in Wisconsin were completed in 1989, and are reported in Reference 28. The following paragraphs briefly describe the monitoring methods and results obtained through the spring, summer, and fall of 1990 in Michigan.

A variable-width line transect method is used to census the bird community. Study sites now consist of 10 transects (five treatment and five control) in Wisconsin and another 10 in Michigan. Treatment transects are parallel to and about 125 m from the edge of the antenna ROW. Control transects are variously oriented and generally at distances greater than 10 km from the antenna. For analytical purposes each transect is subdivided into eight segments. Observers walk along a randomly designated transect to determine bird species and numbers from sightings or bird songs. To properly qualify the collected data, habitat structure on treatment and control transects, possible "edge effects" caused by the antenna ROW, and variability due to differences between observers have been examined.

Since 1986, the identification and enumeration of bird species has been performed during each of five periods throughout the year: spring migration (May), early breeding (June), late breeding (July), early fall migration (August), and late fall migration (September). Parameters selected for statistical analyses include species richness, total number of birds, number of individuals for abundant species, number of individuals for common species, and number of individuals within selected guilds.

It is important to recognize that treatment and control transects (i.e., relatively long, narrow plots) are used in this study, but comparisons between treatment and control transects are not the principal means of analyzing data. Habitats differ over a wide range among bird species, and it is virtually impossible to find paired plots because of habitat preferences. Therefore, results obtained along treatment transects are compared with each other, and results from control transects are compared with each other. Since dissimilarities between sets of treatment data and control data are known for preoperational years, any post-operational changes in the dissimilarities could be attributed to some environmental change, including changes in the EM environment. The 1990 season represents the first full operational year for the NRTF-Republic.

Bird Abundance and Species Diversity. Bird abundance and species diversity were highest between May and July, 1990, and approximately the same along treatment and control transects. More diverse species occupied the control transects only during June and September. As was the case in previous years, a considerable variation was observed in numbers of individuals and species.

The most abundant species vary with the season. When all seasons were combined for 1990, only 5 of 24 comparisons (21 percent) between treatment and control transects were significantly different. Control transect abundance was greater for two of the five cases. These results are consistent with abundance findings for preoperational years.

Prominence of Common Species. Only 17 of 114 treatment-control comparisons (15 percent) of the prominence of species common to the region were statistically significant in 1990. Prominence values were higher for control transects in 10 of the 17 significantly different comparisons. Few species were consistently more abundant along either treatment or control transects. Habitat characteristics accounted for most of the observed differences. The 1990 results were much like those of preoperational years.

Analysis of Guilds. Bird species are classified according to groups called guilds. Species within a guild exhibit similar foraging behavior and preferred breeding habitat. The 1990 guild analysis revealed few significant differences with regard to abundance within guilds between treatment and control transects. The most consistent differences were the abundance of birds that prefer deciduous forest habitats. The 1990 results of guild analysis therefore showed that habitat preferences and differences were primarily responsible for bird distribution patterns in the first year of NRTF-Republic operations. The same conclusion was drawn for preoperational years.

2.8 AQUATIC BIOTA

Aquatic biota, particularly fish, have been shown to use, or react to, weak ELF EM fields. The purpose of this study is to monitor a riverine ecosystem for possible effects to aquatic biota from long-term exposure to the low-level EM fields produced by the ELF Communications System in Michigan. Populational aspects as well as the functional and structural components of three major aquatic communities (periphyton, aquatic insects, and fish) are examined.

Two similar sections of the Ford River are used as matched study sites. One site--the treatment site--is located adjacent to the north-south leg of the NRTF-Republic; the control site is located more than 10 km downstream. No major tributary exists between the sites. At each site, ambient environmental factors are monitored and ecological experiments occupy adjacent stream segments. Four additional netting sites are located upstream of the control site in order to determine the migration patterns of fish.

2.8.1 Periphyton

Periphyton are a community of microscopic plants and animals that attach to and live on the surfaces of submerged objects. The structural and functional aspects of the periphyton community at a given location, unlike those of organisms suspended in water, are governed by conditions at that point. Because they show responses immediately at the source of a perturbation, periphyton are being used to assess possible changes in the aquatic community due to the operation of the ELF Communications System.

Since the periphyton community is dominated by diatoms, these plants are emphasized in monitoring structural aspects; however, functional aspects such as chlorophyll, biomass, photosynthesis, and respiration are determined for the entire community (i.e., diatoms, plants other than diatoms, and animals). Quantitative determinations are made by collecting periphyton colonizing artificial substrates of a known surface area. Preliminary studies in the Ford River have shown that the periphyton established on glass slides are representative of the periphyton community found on natural substrates.

Statistical comparisons between sites used the paired t-test; however, "before and after, control and impact site" (BACI) techniques were emphasized in most analyses. The BACI technique compared the mean of the "before" differences between control and impact (treatment) sites to the mean of the "after" differences between sites by using an unpaired t-test. For preliminary statistical analyses, samples collected from June 1983 through April 1986 are considered "before" data and samples collected from May 1986 through September 1990 are considered "after" data. Low-current testing was initiated at the NRTF-Republic during July 1986, and full-time operations commenced in late 1989.

The purpose of this element is to monitor selected variables of the diatom component of the periphyton community. Indices for species diversity, evenness, and abundance allow the detection of subtle shifts in the community's structural makeup; total cell density and biovolume of diatoms give an indication of any overall change in the dominant biota of the community.

Glass slides emplaced at study sites for 28 days were used to identify and enumerate colonizing diatoms. The community that develops on emplaced slides most often consists of 50 to 70 species of diatoms. Because diatoms vary greatly in their size distribution, the number of individuals (total cell density) alone does not adequately describe the community's makeup. Therefore, cell volume measurements for the dominant diatoms were also determined. Volume estimates were multiplied by the density of each species and summed to provide an estimate of total biovolume.

Because structural characteristics alone do not provide a complete characterization of the periphyton community, the purpose of this element is to monitor functional aspects such as chlorophyll a, organic matter accumulation, photosynthesis, and respiration, all of which represent the functioning of the entire community.

Slides were emplaced in the Ford River for 14 days for determinations of accrual rates, and for 28 days for determinations of standing crop estimates of chlorophyll *a*, phaeophytin *a*, and organic matter biomass. Fluorometric methods were used for analyses of chlorophyll and phaeophytin. Organic matter biomass was determined using changes in ash-free dry weight per unit area.

The data obtained prior to the summer of 1986 provide a good description of diatom dynamics. The chlorophyll *a* standing crop and accrual at the Ford River sites exhibits a pattern of July-August highs and winter lows. There is considerable variability in the data from year to year. Organic matter standing crop exhibits the same seasonal characteristics. Spring weather conditions have a large influence on yearly variability.

The seasonal pattern for species diversity and evenness at the Ford River sites has also been well-established. Diversity and evenness are higher during winter than during summer. Diatom density, however, is lowest during winter. The peak density has consistently occurred within a four-month spring-summer period. The time and duration of the peak is highly variable from year to year during that period. Water temperature is an important influencing factor.

Paired t-tests of data collected through 1990 show that diatom dynamics at the treatment and control sites remain essentially the same. The few slight differences that have been found have no biotic significance.

The BACI statistical tests of data have shown that there are some statistically significant differences in some parameters. The differences have been correlated with grazing invertebrates and weather variables that have been recorded yearly.

Since diatom dynamics have not changed between sites and only yearly changes have occurred, it seems apparent that ELF System EM fields have not had an influence on the periphyton community to date.

2.8.2 Aquatic Insects

As part of the integrated studies of the aquatic ecosystem, insects are being monitored as representative of the primary and secondary consumer levels in the aquatic food chain. These studies examine the important functional insect groups, such as shredders, collectors, predators, and grazers. Both community and individual aspects of organization are being monitored. The community aspects are leaf litter processing; insect colonization patterns on leaf litter and artificial substrates; and structural descriptors of community change such as species richness, individual abundance, and species diversity. Individual aspects emphasize changes in behavior such as alterations in movement patterns and feeding activity.

Feeding Activity of Grazers. The movement of energy from producer (periphyton) to higher trophic levels is important in maintaining the aquatic community. The relationship of a grazing insect to the periphyton community is being monitored to determine possible effects from the ELF System on energy transfer.

The study approach uses streamside chambers to which are added tiles precolonized with periphyton and grazing insects. The chambers are subdivided to allow different numbers of grazers (0 to 30) per experimental run. The tiles are removed after a period of time, and the periphyton are analyzed for chlorophyll *a*, organic matter biomass, and diatom cell counts. Development of data collection techniques began in 1985 and continued into 1989.

Preliminary studies have shown that a grazing insect can change the composition of the periphyton community at the grazer and diatom densities found naturally in the Ford River. Grazing, however, does not affect all community parameters (e.g., chlorophyll *a*, or biomass), and its effects are not consistent. Studies performed in 1985 and 1986 showed that grazing affected the abundance of major species, yet in 1987 there were no measurable changes between grazed and ungrazed tiles, and abundance changes for major species in 1988 were opposite those determined in 1985 and 1986.

Researchers attributed the lack of effects between grazed and ungrazed tiles to short exposure times and silting problems encountered during the course of the studies. In 1989, monitoring experiments were extended from 7 days to 14 days, and silting problems were not encountered.

Benthic Insect Community. Studies of possible EM effects on aquatic insects cannot be found in the literature; however, effects of high-intensity ELF EM fields on the behavior of terrestrial insects have been documented. It has also been reported that some aquatic biota use weak EM fields to locate prey or orient themselves. Benthic insects being major organisms in the primary and secondary trophic levels of the Ford River, their community structure and function is being monitored for possible effects from operation of the ELF Communications System.

Riverine substrates contained in sample baskets were emplaced at study sites for one-month periods at intervals throughout the spring, summer, and fall seasons. Insects were collected from the substrates, identified, and counted. Numbers of individuals, diversity, richness, evenness, and percent numerical dominance for selected species were determined for each replicate. Total sample biomass and biomass for functional feeding groups were determined. For those insects with high numerical abundance, mean dry weight per individual was also computed.

Leaf Litter Processing. In headwater streams such as exist in the ELF System area, only a small portion of the energy supply to the ecosystem is provided by aquatic plants and algae. The maintenance of community structure is largely dependent on the input of organic materials (i.e., leaves) from riparian vegetation. Macroinvertebrate consumers, mainly insects, process the leaves, making the leaf biomass available to higher trophic levels (predators).

Processing and insect colonization patterns using "leaf pack" bioassay techniques were used to monitor for possible EM effects to this energy pathway. Leaf processing rates (mass loss) were used to quantify the overall feeding activities of the colonizing community, while species diversity, evenness, and richness were used to characterize colonization patterns. Processing rates were determined for both fresh and autumn-abscised leaves emplaced in the Ford River and retrieved at regular intervals over an 80-day period each year. Leaf processing rates were computed as decay coefficients and analyzed using Wilcoxin rank sum tests for intersite comparisons. Fresh and autumn leaf data were analyzed separately.

Results to Date. Insects associated with the stream bottom of the Ford River exhibit distinct seasonal population and community patterns. Data obtained prior to initial operation of the ELF Communications System were sufficient to describe those patterns adequately. Additionally, the observed variations in patterns could be related to stream and ambient weather conditions. Several examples of findings are summarized below.

The variations in structural and functional community parameters for benthic insects in the Ford River were lowest during the summer and highest during the spring and fall; therefore, data were separated by season for analysis. ANOVA showed significant differences between sites, between years (1984-1989), and year-site interactions for many of the community parameters. Those parameters showing significant differences between years and year-site interactions were further examined using BACI tests. The BACI tests examined for significant differences before (1983-1985) versus after (1986-1989) full-power ELF System operation. Results for the summer data sets (the period most likely to indicate an effect) showed no significant differences in species diversity, evenness, species richness, number of individuals, total insect biomass, or predator/prey ratios. The BACI tests showed some differences in these parameters for the spring and fall periods; however, most of the differences were correlated with physical factors. At present, researchers attribute most statistically significant differences in community parameters to factors other than ELF EM fields.

Analysis of leaf litter processing data has shown that fresh leaves are processed by aquatic organisms faster than leaves falling in the autumn. The processing rates of fresh leaves were about the same each year at the two sites, but the rate changed from year to year. The processing rates of autumn leaves also exhibited changes from year to year; however, processing was consistently faster each year at the treatment site. The explanation for these site differences appears to be that autumn leaves at the treatment site support a more diverse community of aquatic insects.

When analyzed for the year alone, the data obtained during 1990 exhibited much the same characteristics as data from previous years. Few differences between sites were found, and those differences were like those observed in earlier years. There were variations of a yearly nature when the data were analyzed on a year-by-year basis. Such yearly variations also have occurred in the past, and can be related to stream discharge values and cumulative degree-days. There are, nevertheless, suggestions of a before- and after-ELF-activation trend in the data.

2.8.3 Fish

Fish are tertiary consumers feeding on other, lower trophic levels. Possible effects at the producer level (diatoms), primary consumer level (grazers, shredders), or secondary consumer level (insect predators, small fish) can be reflected in the community structure of the tertiary consumers. In addition, some species of fish have an ability to perceive extremely weak EM fields. Since it has been shown that fish use this perceptive ability to orient themselves and to detect prey, the structure of the fish community and movement characteristics of the mobile fish community are being monitored for possible effects from the operation of the ELF System.

Mobile Fish Community. Fyke nets and weirs have been deployed across the width of the Ford River drainage at five sites in or near the ELF Communications System. All fish are collected, and both community characteristics and movement through the area are recorded. Community characteristics recorded are species composition, species abundance, and biomass. The travel time of marked fish through the ELF System area, as well as the condition of abundant fish species, are also examined.

To assess possible direct effects of the ELF Communications System on the mobile fish community, analyses were initiated in 1986 for determining the growth and condition of captured fish. The common shiner, creek chub, white sucker, and northern pike were selected as indicator species for the community. In addition to growth, the condition of common shiners, creek chubs, and white suckers was examined using relative weight condition factors.

Brook Trout Movement. The purpose of this element is to monitor for possible effects to an important game fish, the brook trout. Attributes being monitored include pattern, rate, and magnitude of migration, as well as population aspects such as age, growth, and condition. The general pattern of trout migration has been an upstream movement in the spring to early summer, with a varied intensity and timing of peak movement from year to year. Trout migrate through the ELF System area (control and treatment sites) to the confluence of the Ford River and Two Mile Creek. Virtually all trout migrate up Two Mile Creek; optimal growth temperatures appear to be responsible for this movement. No downstream movement from Two Mile Creek has been observed for sampling periods lasting through November. Factors affecting timing of peak catches and distribution patterns appear to be water temperature, stream velocity, and trout population size.

Results to Date. The number of species collected at the downstream control sites has been higher each year than the number collected at the treatment site. Species diversity between sites has not been significant, however. Typically, only a few of the less common species have been found at the control site. Five species have consistently accounted for the fish biomass each year since monitoring began. They are the common shiner, creek chub, *burbot, brook trout, and white sucker.* Biomass has varied from year to year, but no significant differences have been found between sites.

The movement of fish through the portion of the Ford River in the ELF System area also has been quite variable. Movement is especially slow during dry summers, several of which have occurred since fish movement studies were initiated.

Data collected to date have shown that shiners and creek chubs in the Ford River tend to be larger than the sizes reported in the literature as average sizes. On the other hand, white suckers and the less common northern pike are relatively small. Size in itself is not necessarily a good indication of the status of the fish community. The condition of common shiners has been found to be above average, but the condition of creek chubs and white suckers has been below mean values reported in the literature. Brook trout, important game fish in the Ford River, are relatively large, but generally do not weigh as much as the weights reported in the literature as average weights.

Two years of operational data (1989 and 1990) have now been obtained and analyzed. Thus far, there is no indication of ELF EM effects on species diversity, biomass, daily rates of movement of brook trout, or brook trout size and condition. Size and condition of other species common to the Ford River also appear to be unaffected. However, as was also reported for aquatic insects, there has been some suggestion of behavioral modifications (indicated by brook trout recapture data) since 1988. Several more years of monitoring are necessary to determine if behavioral modifications are indeed occurring, and if so, whether those modifications are due to some natural environmental condition or to ELF EM fields.

3. ENGINEERING SUPPORT

The relationship between low-level EM exposure and biological response remains unresolved. Beyond EM intensity comparisons (treatment and control, before and after), other EM characteristics of the ELF Communications System may need determining to further interpret the results of the biological studies. For example, EM aspects of potential concern are:

- exposure duration
- duty cycle (power, number of "on/off" powerings)
- interaction with other fields
- wave shape
- phasing between antenna elements

This is not to say that these aspects will cause biological effects, but only to indicate the need to document various facets of the EM fields generated by the ELF System while the monitoring program is in progress. Because extensive and accurate EM data may be needed at a later time to fully evaluate cause-and-effect relationships between EM exposure and biological/ecological end points, IITRI assists university investigators by providing them with engineering support.

The following subsections summarize the operational characteristics of the ELF System and measurement of EM field exposures at study sites, as well as other engineering activities carried out by IITRI in support of the monitoring program during 1990. A more extensive presentation of these topics can be found in Reference 2.

3.1 TRANSMITTER OPERATIONS

Data on transmitter operations in Michigan have been provided to IITRI by the Navy on a minute-by-minute basis, and included all changes in operational frequency, modulation, power, and phasing for each antenna element. This information has been received as graphical and tabular summaries and, when requested, in detailed tabular form. The operational history of the ELF Communications System in Michigan prior to 1989 has been summarized in Reference 2. The NRTF-Republic became fully operational in October 1989, and during 1990 it operated according to its design objectives, as follows:

- 150 amperes antenna current
- 76 Hz
- MSK modulation
- 8200 hours of operation (93.5 percent of the time)

3.2 EM FIELD MEASUREMENTS

EM field measurements are made at ecological study sites (test and control plots) at least yearly, and more often, if necessary, to characterize EM environments. Characterizations are needed to support analyses of study results obtained by ecological research teams because EM fields are the environmental influence of principal concern. Measurements are made at 76 Hz, the operating frequency assigned to the ELF Communications System; 60 Hz EM fields, produced principally by electric utility systems, also are measured.

EM field measurements were made in 1990 during the months of January, May, June, August, September, and October. Data were obtained at 50 study sites of interest to ecological research teams. No new sites were established in 1990. However, an additional 15 sets of data were obtained to better characterize EM field distributions at several study sites. Data sets therefore increased during 1990 from a total of 187 to 202.

As expected, some changes in 60 Hz EM fields were observed in 1990 relative to data obtained in previous years. The changes were attributable to consumer usage patterns and changes in commercial electric power systems. EM fields at 76 Hz in 1990 were consistent with those expected from full-time, full-power operation of the NRTF-Republic. Measurement protocols, instrumentation, calibration, and resulting data are documented in detail in Reference 2.

3.3 OTHER SUPPORT ACTIVITIES

In addition to measuring and interpreting EM field characteristics at study sites used by ecology investigators, IITRI engineers and technicians also provide other support requested by investigators. Support during 1990 is discussed in detail in Reference 2, and is summarized below.

Investigators involved with small mammals and nesting birds must, from time to time, remove some animals from field sites to a laboratory at Crystal Falls, Michigan.

Measurements of 76 Hz and 60 Hz EM fields were made at the Crystal Falls laboratory during 1990, as in previous years, to ensure that criteria established for the Ecological Monitoring Program were satisfied at the facility.

Nest blocks used by native bees also are transferred from field sites to the Crystal Falls laboratory from time to time. EM fields at the bee-holding facility were measured during 1990, as in previous years.

Research teams involved with bird species and community studies requested additional measurements to identify variations in EM fields along the relatively large transects (200 m by 4500 m) used in their studies. The additional measurements were made during 1990, and observed spatial variations were interpreted to assist investigators in analyzing study results.

Researchers studying upland flora and soil microflora requested additional data regarding temporal and spatial variations at treatment plots in 1989. Additional data were obtained during 1989 and 1990 at the sites of interest. EM field contours have been developed to aid researchers' analyses of study results.

Sealed culture cells are used at soil amoeba study sites. Since electric field intensity is affected by soil conductivity, it is necessary to monitor electric fields at the sites to account for seasonal changes in soil conductivity (a weather-related phenomenon). As in previous years, data were obtained in 1990 to describe temporal changes in culture cell electric fields and to relate these changes to electric field variations in surrounding soils.

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APPENDIX A

ECOLOGICAL MONITORING PROGRAM: LIST OF PUBLICATIONS/PRESENTATIONS, 1982-1990

ECOLOGICAL MONITORING PROGRAM: LIST OF PUBLICATIONS/PRESENTATIONS, 1982-1990

Upland Flora (Michigan Technological University)

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Small Mammals and Nesting Birds (Michigan State University)

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21. Stout, R. J. Comparison between fresh and autumn dried leaf inputs in two deciduous forest streams. Presented to the Entomological Society of America; 1983; Detroit, MI.

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1. Marod, S. M.; Taylor, W. W. Temperature initiated brook trout movements in the Ford River, Dickinson County, Michigan. Presented at the 51st Midwest Fish and Wildlife Conference; 1989; Springfield, IL.

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4. Mullen, D. Long nose dace in the Ford River, Michigan. Presented to the North American Benthological Society; 1989; Guelph, Ontario, Canada.

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15. Zapotosky, J. E. ELF Communications System Ecological Monitoring Program. Presented at the Eighth Annual Meeting of the BioEMs Society, Madison, Wisconsin, 1986.

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Annual Progress (IIT Research Institute/Subcontractors)

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3. Compilation of 1988 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06595-6, Vol. 1, 572 pp.; Vol. 2, 351 pp.; Vol. 3, 419 pp.; 1989.

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5. Compilation of 1987 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06595-2, Vol. 1, 706 pp.; Vol. 2, 385 pp.; Vol. 3, 491 pp.; 1988.

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7. Compilation of 1986 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06549-38, Vol. 1, 445 pp.; Vol. 2, 343 pp.; Vol. 3, 418 pp.; 1987.

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9. Compilation of 1985 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06549-26, Vol. 1, 472 pp.; Vol. 2, 402 pp.; Vol. 3, 410 pp.; 1986.

10. Ecological Monitoring Program, ELF Communications System: 1986 Technical Symposium, IIT Research Institute; 1986; Escanaba, MI.

11. Compilation of 1984 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program. IIT Research Institute, Technical Report E06549-17, Vol. 1, 528 pp.; Vol. 2, 578 pp.; 1985.

12. Ecological Monitoring Program, ELF Communications System: 1985 Technical Workshop, IIT Research Institute; 1985; Cable, WI.

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14. Ecological Monitoring Program, ELF Communications System: 1983-1984 Workshop, IIT Research Institute; 1984; Roscommon, WI.

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APPENDIX B

ECOLOGICAL MONITORING PROGRAM: FY 1990 RESOURCES

ECOLOGICAL MONITORING PROGRAM: FY 1990 RESOURCES

The Navy has been committed to a program of long-term ecological monitoring since the ELF Communications System site selection process was initiated. The Ecological Monitoring Program is identified separately from other environmental protection work for future year budgeting purposes; therefore, continuity of the program is anticipated, presuming continued Congressional approval and funding of the ELF Communications System.

During 1990, monitoring studies were conducted under eight subcontracting agreements between IITRI and study teams from three universities (see Table B-1). IITRI provides engineering support and overall program management. Each study team is headed by a principal investigator with academic training to the doctoral level. Most of the staff also have advanced degrees, with expertise and publications in the areas under study. During 1990, the Ecological Monitoring Program consisted of more than 90 people expending a total of about 86,000 staff hours.

TABLE B-1. ECOLOGICAL MONITORING PROGRAM: FY 1990

Study	Subcontractor	Principal Investigator(s) (Total Staff)	Professional and Staff Hours 1990
Upland Flora	Department of Forestry Michigan Technological University	G. D. Mroz, Ph.D. (18 persons)	15,589
Soil Microflora	Department of Forestry Michigan Technological University	J. N. Bruhn, Ph.D. (7 persons)	4,510
Soil Amoebae	Department of Zoology Michigan State University	R. N. Band, Ph.D. (6 persons)	5,287
Soil Arthropods and Earthworms	Department of Zoology Michigan State University	R. J. Snider, Ph.D. R. M. Snider, Ph.D. (15 persons)	14,534
Native Bees	Department of Entomology Michigan State University	K. Strickler, Ph.D. M. Scriber, Ph.D. (10 persons)	7,360
Small Mammals and Nesting Birds	Department of Zoology Michigan State University	D. L. Beaver, Ph.D. (10 persons)	12,592
Bird Species and Communities	Natural Resources Institute University of Minnesota-Duluth	G. J. Niemi, Ph.D. J. M. Hanowski (9 persons)	6,288
Aquatic Biota	Departments of Zoology, Entomology, Fisheries and Wildlife Michigan State University	T. M. Burton, Ph.D. R. J. Stout, Ph.D. W. W. Taylor, Ph.D. (15 persons)	14,8[illegible]1
Program Integration and Engineering Support	EMs and Electronics Department IIT Research Institute	J. E. Zapotosky, Ph.D. (4 persons)	5,200
		TOTAL	**86,241**